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Life History Analysis of James River Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*) with Implications for Management and Recovery of the Species

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Title

Life History Analysis of James River Atlantic Sturgeon (*Acipenser
oxyrinchus oxyrinchus*) with Implications for Management and
Recovery of the Species

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of
Philosophy at Virginia Commonwealth University.

by

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Abstract

Sturgeon species (family Acipenseridae) are threatened globally due to habitat destruction, pollution, and overfishing. The Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* was listed as a federally endangered species in 2012. Atlantic sturgeon have a complex life history that utilizes a wide range of habitats. Timing of life history varies in different areas requiring each population to be studied. Very little work has been published on Atlantic sturgeon reproduction in the James River, Virginia. To aid the recovery of James River Atlantic sturgeon, aspects of life history need to be addressed. To increase understanding of Atlantic sturgeon life history a length at age model was created to show population structure and age of sexual maturity. Telemetry experiments were conducted to determine what types of boats are likely to cause boat strike mortalities of adult fish during a spawning season. Morphometrics, sperm characteristics, and telemetry data were used to determine if Atlantic sturgeon have a fall spawning season. Strontium/calcium ratio analysis was conducted on Atlantic sturgeon fin spines to better understand migration patterns. Cortisol levels were examined in Atlantic sturgeon exposed to MS222, electronarcosis or no anesthetic 1 and 24 hr after a small incision mimicking tag implantation. I also determined the feasibility of using electronarcosis in the field and the effect of salinity on electronarcosis. The length at age data show male Atlantic sturgeon become sexually mature at age 10 y and females around age 15 y. Telemetry data showed that deep draft ocean-cargo ships are most likely responsible for boat strike mortalities and there is a greater chance of Atlantic sturgeon being hit in the narrow portion of the river. Electronarcosis is an effective anesthetic and has various attributes that make it better suited for field applications than frequently used chemical anesthetics. The data generated from this research

will help management produce effective recovery plans and create a safer research environment for both the fish and researcher.

Key Words

Atlantic sturgeon, ship-strikes, growth, electronarcosis, cortisol

Introduction

Dam construction, pollution, habitat alteration, and overfishing have decimated sturgeon species (Acipenseridae) throughout the world (Bain 1997; Birstein et al. 1997; ASSRT 2007; Hatin et al. 2007). Demand for Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) roe and flesh during the 19th century promoted a major fishery along the Atlantic coast of U.S. and Canada that was profitable for about half a century (Ryder 1890; Cobb 1900; Tower 1908; Hildebrand and Schroeder 1928). The largest reported catch, an estimated 3.4 million kg, was harvested from the Atlantic coast of the U.S. in 1890. Due to unsustainable harvest, all major U.S. Atlantic sturgeon fisheries collapsed by the early 1900s (Hildebrand and Schroeder 1928; Smith 1985). By 1901 national landings were only 10% of the record 1890 landing (Figure 1).

Currently, Atlantic sturgeon range from St. John River, New Brunswick to the St. Johns River, Florida (Murawski and Pacheco 1977). Historically Atlantic sturgeon inhabited the Baltic Sea but was extirpated from that area (Ludwig et al. 2002; Ludwig et al. 2008). Prior to extensive harvest Atlantic sturgeon were abundant in many rivers of the U.S. Eastern sea-board (Ryder 1890; Hildebrand and Schroeder 1928; Smith and Clugston 1997). Of the 35 rivers in which Atlantic sturgeon reproduced historically, only 20 river systems currently have confirmed spawning. However, all confirmed spawning rivers currently have critically depleted Atlantic sturgeon numbers (ASSRT 2007) and most are not recovering. Many aspects of the Atlantic sturgeon's life history may be hindering recovery. Understanding life history is vital for creating effective management strategies to help restore species existing at severally reduced levels. After a detailed life history analysis of a hindered species is completed management will be able to create a better recovery program (Waldman and Wirgin 1998; Limburg and Waldman 2009).

Atlantic sturgeon have been aged up to 60 y (Magnin 1964) and measured up to 4.5 m total length (Scott and Crossman 1973). However, both of these measurements are likely underestimates because the samples were taken decades after the fishery collapsed. Both Ryder (1890) and Smith (1985) provide accounts of fish almost 6 m long. Atlantic sturgeon are an iteroparous-anadromous species making them vulnerable to human exploitation (Boreman 1997, Birstein et al. 1997). Their anadromous life history requires access to various habitats which can be cut-off by dam construction (Smith 1985; ASSRT 2007). Timing of life history stages varies clinally with southern populations growing and maturing faster than northern populations (Smith 1985, Bain 1997, Balazik et al. 2010). Atlantic sturgeon are late maturing. Males from the South Carolina area mature around 8 y and females around 11 y, Hudson River males mature around 11 y and females around 20 y, and St. Lawrence River males mature around 22 y and females around 27 y (Scott and Crossman 1973; Dovel 1979; Smith 1985). The slow sexual maturity of Atlantic sturgeon adds to recovery problems due to long generation times compared to many other fish species (Bemis and Kynard 1997; Bemis et al. 1997). Even though Atlantic sturgeon are iteroparous; individuals do not spawn annually (Bain 1997; Kynard and Horgan 2002). Smith (1985) describes marks on fin spines suggesting females spawn every 3-5 y and males spawn 1-5 y. However new evidence suggest males probably spawn every 1-2 y (Collins et al. 2000).

Historically Atlantic sturgeon spawning occurred in spring, with southeastern populations (Georgia-Carolinas) spawning as early as February. Spawning occurs in the mid-Atlantic (Virginia-Delaware) in April and in northern regions (New England-St. Lawrence) spawning was as late as July (Vladykov and Greeley 1963; Bigelow and Schroder 1953; Scott and Crossman 1973). The driving force to initiate spawning runs seems to be water temperature (Smith 1985)

with optimum water temperatures being 20-21°C (Mohler 2004). Atlantic sturgeon have demersal-adhesive eggs that require hard-clean substrate for successful reproduction (Ryder 1890; Dean 1893; Dean 1894; Cobb 1900; Dees 1961; Leland 1968; Smith et al. 1980; Collins et al. 2000; Mohler 2004). Lack of hard-clean substrate due to sedimentation hinders reproduction success (ASSRT 2007).

Spawning typically occurs in groups with two or more males to one female. A female will position herself about 0.5-1.0 m above proper substrate while males move under and rub/bump the female's ventral side to stimulate egg release (Ryder 1890). Once the eggs are broadcast the males release sperm (Ryder 1890). The fertilized eggs then adhere to the substrate and develop (Smith 1985; Mohler 2004). Fertilized egg development is water temperature dependent with eggs hatching at 2 d (24°C), 4 d (20°C), and 6 d (17.5° C) (Dean 1894; Smith et al. 1981; Mohler 2004). Post-hatch larvae hide in benthic interstitial spaces for about 8-9 days before beginning exogenous feeding (Ryder 1890; Smith et al. 1981). Not much is known about young-of-year Atlantic sturgeon movements (ASSRT 2007); however, findings suggest they congregate within a few river kilometers (rkm) upstream of the estuary saltwedge (M. Fisher, Delaware Department of Natural Resources, unpublished data). The timing of Atlantic sturgeon movements beyond age-1 y is generalized from mark-recapture and tracking data. At age-1 Atlantic sturgeon migrate to the brackish-estuarine zone and coastal areas and remain there until around age 6 y. After age 6 y the fish tend to move into the open ocean until they return to their natal river to spawn (Bain 1997; ASSRT 2007)

Spawning river fidelity has an enormous potential impact on Atlantic sturgeon management. Strong natal river fidelity is inferred from tracking data, mark-recapture data (Smith et al. 1982) and genetic studies. Using mitochondrial DNA, at least 14 distinct

population segments (DPS) have been recognized among Atlantic sturgeon (Wirgin et al. 2000). Using microsatellite DNA analysis only six DPS have been recognized (Figure 2): St. Lawrence, Gulf of Maine, N.Y. Bight, Chesapeake Bay, Carolinas, and South Atlantic (King et al. 2001). The National Oceanic and Atmospheric Administration (NOAA) bases management following the six DPS determined from microsatellite DNA analysis (ASSRT 2007). In 2012 all DPS were listed as either threatened or endangered under the Endangered Species Act with the Chesapeake Bay DPS classified as endangered. Although Atlantic sturgeon have been studied for over a century along their range very little work has been published for the Chesapeake Bay region.

Chesapeake Bay Atlantic Sturgeon

During peak harvest in the late 19th century, the Chesapeake Bay region was the second largest producer of Atlantic sturgeon products (Smith 1985; Secor 2002). Most tributaries of the Chesapeake Bay were utilized by adult Atlantic sturgeon; however, the major fishery operations were conducted in the James, York, Rappahannock, and Potomac Rivers. In 1880 Potomac River landings were 130,000 kg, more than double the 50,000 kg taken from the James River. The York River accounted for 23,000 kg and the Rappahannock only 8,000 kg. By 1920 Chesapeake Bay landings were only 3% (10,000 kg) of the 1890 landings (408,000 kg; Figure 3). Due to the fisheries collapse Virginia State law banned harvest of Atlantic sturgeon less than 1.2 m around 1927 (Hildebrand and Schroeder 1928). Although no major fisheries were still in operation adults were still captured annually, usually in pound nets as bycatch (Hildebrand and Schroeder 1928). Minimal harvesting continued in Virginia during the 1900s, but with lack of recovery the Virginia Marine Resources Commission implemented a moratorium in 1974 on all Atlantic sturgeon harvest in Virginia waters. The Atlantic States Marine Fisheries Commission expanded the moratorium to all U.S. waters in 1998 (ASMFC 1998). Towards the end of the

20th century many researchers considered the Chesapeake Bay population extirpated (Secor 1996; Speir and O'Connell 1996; Colligan et al. 1998).

In July 1996 a reward program was established to collect hatchery juvenile Atlantic sturgeon (n = 3275) released in the Nanticoke River. By the end of the reward program 262 hatchery fish were recaptured (Secor et al. 2000). However, during the reward program small wild Atlantic sturgeon were also collected (Albert Spells, USFWS, pers. comm.). After the reward program ended Atlantic sturgeon captures from watermen continued as bycatch in other fisheries with hundreds of juvenile Atlantic sturgeon being caught in the James and York Rivers (Kelly Place, Virginia Watermen's Association, pers. comm.). Genetic analysis from tissue samples from watermen collections indicated a DPS exists in the Chesapeake Bay (King et al. 2001). Reproduction in the James (2004) and York (2011) Rivers were verified by trawl collections of young-of-year, less than 15 cm fork length (FL), Atlantic sturgeon (Albert Spells, USFWS, pers. comm., Hank Brooks, Virginia Institute of Marine Science, pers. comm.). From 2006 to 2009 over 130 sexually mature Atlantic sturgeon have been collected in the James River above rkm 120. One Atlantic sturgeon (estimated 1.8 m FL) broke out of a net at rkm 132 of the Pamunkey River branch of the York River. Numerous life history aspects of Atlantic sturgeon in the Chesapeake Bay are unknown and increased knowledge will help management practices, specifically to: 1) document population structure; 2) document spawning periods and frequency; 3) determine spawning locations; 4) describe life history stages temporally; 5) document factors that might hinder restoration; 6) use findings to develop a James River Atlantic sturgeon management plan describing future research needs.

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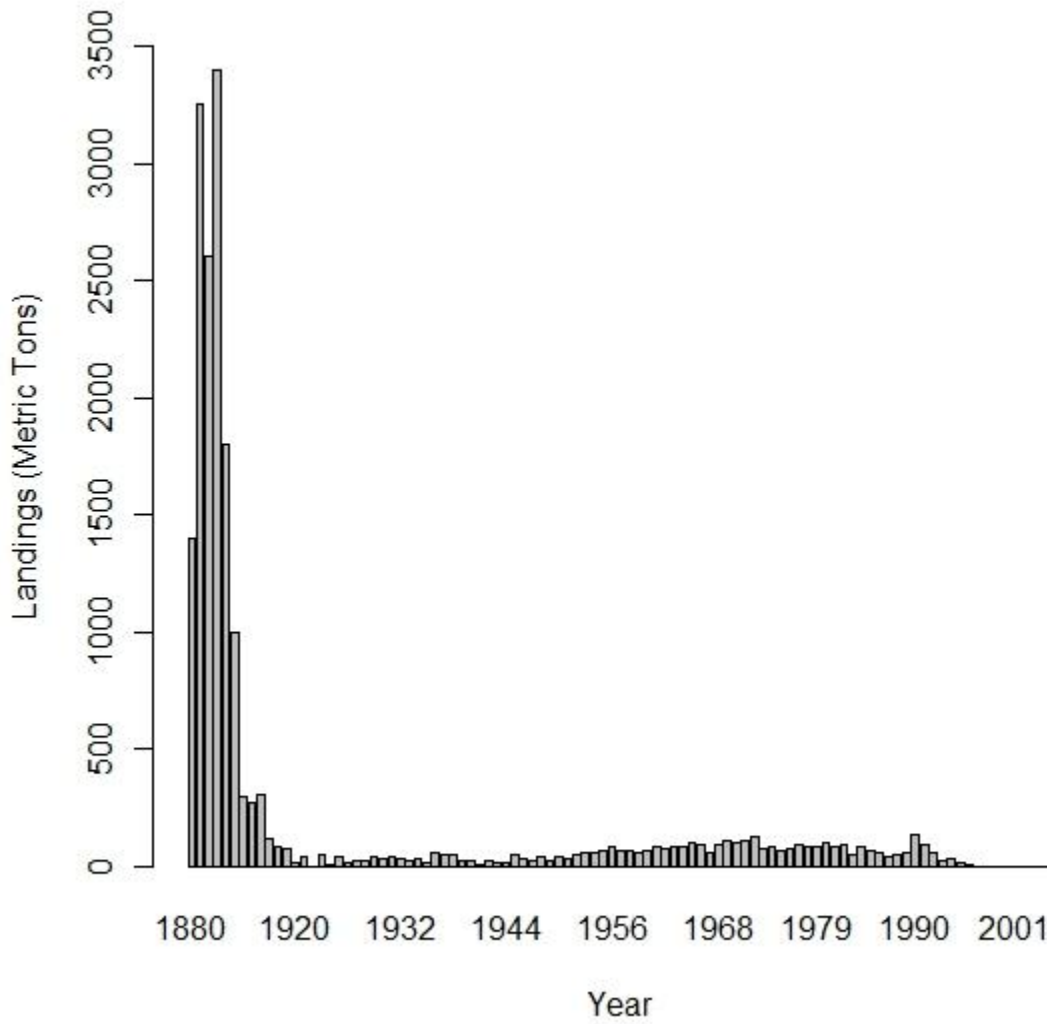


Figure 1. Bar graph showing historical U.S. Atlantic sturgeon landings. Landings values were provided by the National Oceanic and Atmospheric Administration.

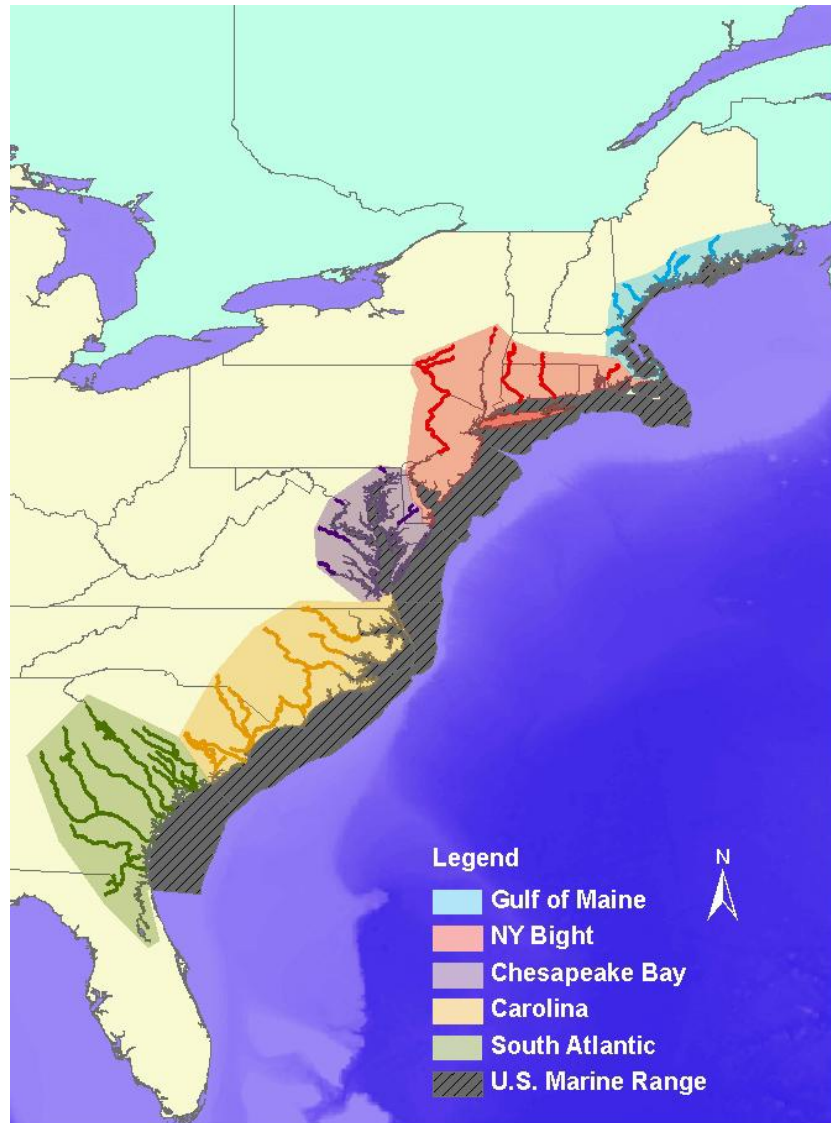


Figure 2. Map showing distinct population segments recognized by the National Oceanic and Atmospheric Administration. Map provided by the National Oceanic and Atmospheric Administration.

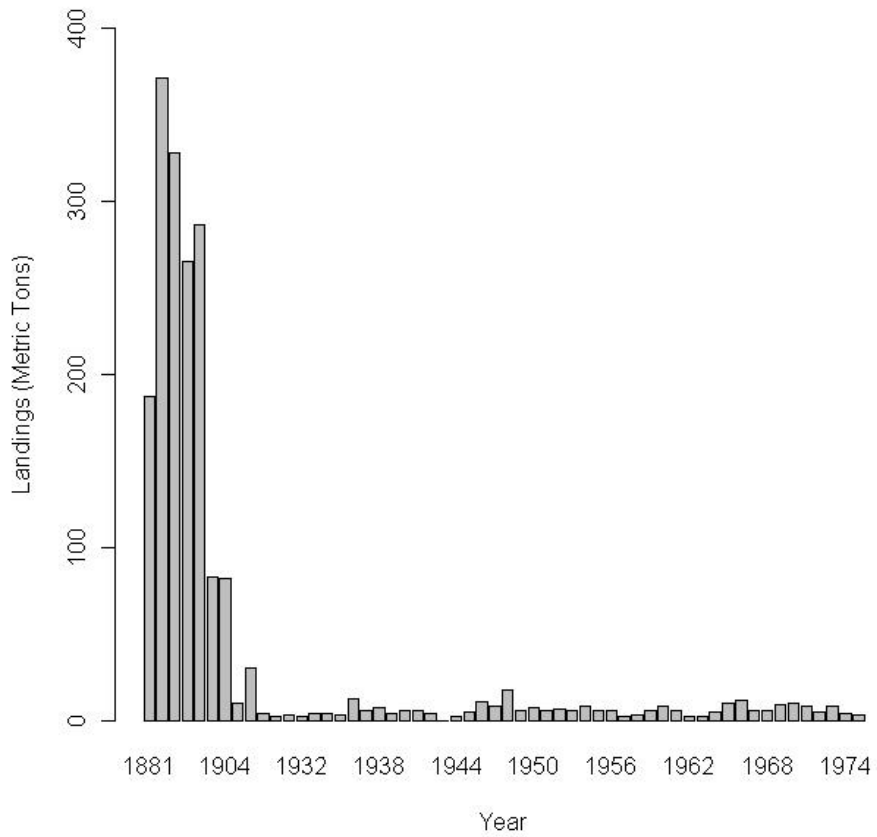


Figure 3. Bar graph showing historical Chesapeake Bay Atlantic sturgeon landings. Landings values were provided by the National Oceanic and Atmospheric Administration.

Chapter I

Age and Growth of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) in the James River, Virginia, USA, 1997-2011

Abstract

Historically the Chesapeake Bay supported a large Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) population, but loss of suitable spawning habitat and overfishing coincided with dramatic insystem declines throughout the 20th Century. Atlantic sturgeon harvest moratoriums were implemented in 1974 for Virginia waters and were expanded coast-wide in 1998. In 1997 researchers became aware that commercial fishers in the James River, a tributary of the Chesapeake Bay, were catching juvenile/subadult Atlantic sturgeon as bycatch in various fisheries. Genetic studies showed that the Chesapeake Bay population has maintained genetic integrity and qualifies as a distinct population segment. Between 2007-2011 almost 150 adults have been caught in the tidal-freshwater portion of the James River during putative spawning runs. Pectoral-fin spines from juveniles/subadults collected in the Burwell Bay (rkm 40) and Cobham Bay (rkm 60) areas and mature adult samples from vessel strikes in freshwater around or above rkm 120 were analyzed to create a length-at-age curve for Atlantic sturgeon in the James River. Five models were used to analyze the data, and the double von Bertalanffy ($k_1=0.054$, $k_2=0.097$, $t_1=-2.85$, $t_2=1.09$, $t_p=6.03$ years, $L_\infty=2241$ mm) provided the best fit to the observed data. We estimated an increase in growth coefficient at t_p , which could be an artifact of low sample size or due to ontogenetic changes in habitat use as older fish spend more time in oceanic waters than younger fish. Atlantic sturgeon in the 6-9 year age range are rarely encountered in the James River compared to younger and older age classes, so a more in-depth analysis of the increased growth coefficient would require ocean sampling.

Introduction

Prior to extensive commercial harvesting in the late 19th century, many tributaries of the Chesapeake Bay supported abundant Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) stocks (Murawski and Pacheco 1977; Smith 1985; Atlantic Sturgeon Status Review Team 2007). After the Virginia fishery collapsed in the early 1900s Atlantic sturgeon continued to be harvested typically as bycatch until 1974 when the Virginia Marine Resources Commission placed a moratorium on harvest. In 1998, the Atlantic States Marine Fisheries Commission (ASMFC) implemented a moratorium on Atlantic sturgeon harvesting for all U.S. waters (ASMFC 1998). Many researchers considered the Chesapeake Bay population functionally extirpated (Secor 1996; Speir and O'Connell 1996; Colligan et al. 1998), resulting in it being a candidate for federal protection under the Endangered Species Act (Federal Register Notice RIN 0648-XZ76).

The James River is the southernmost major tributary to the Chesapeake Bay. Historically, the James River supported a large population of Atlantic sturgeon, but loss of spawning habitat and overfishing coincided with dramatic in-system declines throughout the 19th and 20th Centuries (Smith 1985; Balazik et al. 2010). Observations in the 1990s of subadults caught by commercial fishers as well as rare discoveries of dead adult Atlantic sturgeon in the James River (A. Spells, USFWS, pers. comm.) suggested that Atlantic sturgeon inhabited the river. Genetic studies using samples from the James and York rivers showed that the Chesapeake Bay metapopulation has maintained genetic integrity and is distinct from other coastal populations (King et al. 2001). Successful spawning in the James River was verified in 2004 when a 135 mm fork length (fl) young-of-year Atlantic sturgeon was collected at river kilometer (rkm) 110 (Figure 1). It is currently unknown if the James River is the only river in the Chesapeake Bay that sustains Atlantic sturgeon reproduction. However, the James River is

the only river in the Chesapeake Bay watershed where present day adult Atlantic sturgeon have been collected in freshwater reaches.

Some life history characteristics of the Chesapeake Bay Atlantic sturgeon population, including age at maturity and spawning frequency, may be inferred from latitudinally adjacent populations (Smith 1985). Direct determinations of age structure and growth rates are critical for effective restoration and management of vulnerable populations (Campana 2001). Pectoral fin spines are the preferred method for age determination in sturgeon (Brennan and Calliet 1989), and numerous age and growth studies have been conducted on Atlantic sturgeon (Magnin 1964; Squires and Smith 1979; Smith et al. 1982; Dovel and Bergrenn 1983; Van Eenennaam et al. 1996; Stevenson and Secor 1999), but none for the Chesapeake Bay population. The use of pectoral fin spines to estimate age has been validated for Atlantic sturgeon up to 4+ years of age (Stevenson and Secor 1999) and for lake sturgeon, *A. fulvescens* (Rossiter et al. 1995). Even though this technique has been validated for other relatively young sturgeon, age underestimation of older samples is common and must be taken into consideration (Rein and Beamesderfer 1994; Paragamian and Beamesderfer 2003; Hurley 2004; Whiteman et al. 2004). The primary objective of this study was to use pectoral fin spines to create an empirical length-at-age model for Atlantic sturgeon currently inhabiting the James River.

Study Location

Juvenile/subadult Atlantic sturgeon samples were collected by anchored gillnet from the Burwell Bay (rkm 40) and Cobham Bay (rkm 60) areas in 1997-1998 and 2006-2007. Samples from sexually mature fish were collected in freshwater around or above rkm 120 (Presquile National Wildlife Refuge) during putative spawning runs (Figure 1) between 2006 and 2011.

Methods

Sample Collection

A total of 202 juvenile/subadult (307-1127 mm fl) Atlantic sturgeon were sampled. The 1997-1998 juvenile/subadult samples (n=28) were collected during the October and November months via a reward program targeting sturgeon. The remaining juvenile/subadult samples (n=174) were collected in 2006-2007 by commercial fishers conducting a Fishery Resource Grant targeting Atlantic sturgeon. The Fishery Resource Grant provided samples from February through May and used mesh sizes between 10 cm to 35 cm. The wide range of net sizes was used to ensure a wide range of Atlantic sturgeon size classes was sampled. Atlantic sturgeon thought to be mature were collected during the Fishery Resource Grant but were not used for the study because of the collection area's close proximity to the ocean. Small, approximately 1-2 cm long, spine samples were removed from juveniles within 1 cm of the articulation (Brennan and Calliet 1989; Van Eenennaam et al. 1996; Stevenson and Secor 1999; Sulak and Randall 2002) using bolt cutters or hacksaw. No negative effects from spine section removal have been documented (Collins and Smith 1996). All adult samples (n=30, 1355-2483 mm fl) were mortalities from vessel strikes found in August and September from 2006 through 2011 and were the only samples for which sex was determined in this study. Maturity and sex were determined visually by inspecting gonads (Mohler 2004). Most adult collections were males (n=22) with only one confirmed female. All male gonads were at stage 4 (large lobular white testis), the one female was a post-spawn female that released eggs within 2 weeks (J. Mohler, USFWS, pers. comm.) of collection. Sex and gonad stage of the remaining freshwater collections (n=7) could not be determined due to decomposition but are thought to be mature due to length (1355-2483 mm fl). Atlantic sturgeon are obligate anadromous, highly migratory, and demonstrate site

fidelity (Bain 1997; King et al. 2001). The growth rate of Atlantic sturgeon varies clinally with southern groups growing and maturing faster than northern groups (Smith 1985; Balazik et al. 2010). Collections of adult Atlantic sturgeon in freshwater reaches during putative spawning runs are important because the adults were likely spawned in the James River and not from other populations.

Sample Preparation

Soft tissue on pectoral spine samples was allowed to decay and the spines scraped clean. Some samples were boiled for approximately 5 s to facilitate removal of sticky tissue from the surface of the spine. A section (0.4-0.7 mm) from each spine was removed using an isomet saw with a diamond wafering blade (Brennan and Calliet 1989; Van Eenennaam et al. 1996). The section was mounted on a glass slide with thermoplastic resin (Crystalbond™) and polished using a METASERV 2000 polish/grinder with 1200-grit lapidary film (Veinott et al. 1999). Fish age was estimated by counting annuli following published criteria (Van Eenennaam et al. 1996; Stevenson and Secor 1999; Veinott et al. 1999): an annulus was determined to be the translucent (hypermineralized) zone between two opaque zones. The translucent zones (inferred annuli) in spine sections were defined and age assignments were estimated.

The spines were read blind (fl and capture date unknown) by an experienced reader (reader 1) using a Nikon Eclipse E200 compound light microscope at 40x magnification (Veinott et al. 1999). A supporting reader (reader 2) read 88% of the spines following parameters used by reader 1 to help support findings. Variables such as secondary rays and double annuli were discussed prior to age estimation (Stevenson and Secor 1999). The coefficient of variation (CV) of age estimation was determined using a paired-sample t-test to evaluate precision between readers and bias was estimated following Campana et al. (1995).

Growth Curve analysis

Five empirical growth models were fit to the length-at-age data to facilitate model comparison and identify the best description of the data. The use of ordinary least squares to estimate model parameters requires independent observations, normally distributed length observations at each age, and constant variance of lengths across ages (Quinn and Deriso 1999). For Atlantic sturgeon age classes with more than a few length-at-age observations, histograms of the lengths-at-age were positively skewed and resembled a lognormal distribution. Examination of the residuals from preliminary model fits showed that the magnitude of the residuals increased with age. The general distributional patterns of the lengths at each age combined with heteroscedasticity suggested that a multiplicative error structure was appropriate for the length-at-age data. (Quinn and Deriso 1999; Zuur et al. 2010).

The von Bertalanffy model is widely used to describe sturgeon age and growth (Morrow et al. 1998; Stevenson and Secor 1999; Sulak and Randall 2002). The traditional von Bertalanffy function (von Bertalanffy 1938), log transformed for multiplicative error, has the following form:

$$\log(L_i) = \log(L_\infty) + \log(1 - e^{-k(t_i - t_0)}) + \varepsilon_i \quad (1)$$

where L_i is fork length of individual i at age t , L_∞ is the asymptotic length, k is the instantaneous growth coefficient, t_0 is the theoretical age at length zero, and ε_i is the error term. Four additional models, three of which are variations of the von Bertalanffy function, were fitted because of their added flexibility and, in two instances, because they allow growth in proportion to length to change with age. The double von Bertalanffy model (Condrey et al. 1988; Vaughan and Helser

1990; Porch et al. 2002), which allows the rate at which a fish approaches the asymptotic length to change after some pivotal age, t_p , takes the form:

$$\log(L_i) = \begin{cases} \log(L_\infty) + \log(1 - e^{-k_1(t_i - t_1)}) + \varepsilon_i & \text{for } t_i < t_p \\ \log(L_\infty) + \log(1 - e^{-k_2(t_i - t_2)}) + \varepsilon_i & \text{for } t_i \geq t_p \end{cases} \quad (2)$$

$$t_p = (k_2 t_2 - k_1 t_1) / (k_2 - k_1)$$

where k_1 and k_2 are instantaneous growth coefficients before and after the pivotal age respectively, t_1 and t_2 are age intercepts, and ε_i is the error term. The growth function developed by Porch et al. (2002), which is a generalization of the double von Bertalanffy model in that it allows the growth rate in proportion to length to decrease gradually with age rather than at some abrupt pivotal point, takes the form:

$$\log(L_i) = \log(L_\infty) + \log(1 - e^{-\beta_1 - k_0(t_i - t_0)}) + \varepsilon_i$$

$$\beta_1 = \frac{k_1}{\lambda} (e^{-\lambda t_i} - e^{-\lambda t_0}), \quad (3)$$

where λ is the damping coefficient that governs how the overall growth coefficient changes with increasing age. The other two growth models considered were the Richards function (Richards 1959):

$$\log(L_i) = \log(L_\infty) + \left(\frac{1}{\delta} \right) \log(1 - \delta e^{-k(t_i - t_0)}) + \varepsilon_i \quad (4)$$

where δ is a shape parameter, and the sigmoidal Gompertz function (Quinn and Deriso 1999):

$$\log(L_i) = \log(L_\infty) - \left(\frac{\lambda}{k}\right) e^{-kt_i} + \varepsilon_i. \quad (5)$$

The parameters of all growth models were estimated using nonlinear least squares with the software package R version 2.11.0 (*nls* function, R Core Development Team 2010, Vienna, Austria). Growth models were compared using Akaike's Information Criterion (AIC), (Akaike 1973; Burnham and Anderson 2002), which for growth model m can be written in terms of least squares output as follows (Kimura 2008):

$$AIC_m = n(1 + \log(2\pi \cdot RSS_m / n)) + 2p_m \quad (6)$$

where n is the number of data points, RSS_m is the minimized residual sum-of-squares for model m , and p_m is the number of estimated parameters for model m , including the error parameter.

The most parsimonious model that best balances the tradeoff between fit and number of estimated parameters has the lowest AIC value. Because AIC is on a relative scale, it is often important to calculate AIC differences, which are defined as $\Delta AIC_m = AIC_m - AIC_{\min}$, where AIC_{\min} is the smallest AIC value within the candidate set of models. Generally, ΔAIC_m values between 0-2 are indicative of substantial empirical support for the fitted model and values between 4-7 are associated with models that have less empirical support (Burnham and Anderson 2002).

Results

Age Estimation

Of the 203 samples read by both readers, 70% (n=143) had exact age agreement, 23% (n=46) differed by 1 year, 6% (n=12) differed by 2 years, and 2% (n=2) differed by 3 years. There was an average between-reader difference of 1.3 years when age estimation was discrepant. The CV was 1.8%, and there was a significant difference ($t=7.75$, $p<0.05$) between age determinations by the two readers. The bias plot indicates that reader 1 consistently estimated a higher age (Figure 2). There were three fish estimated to be 10 years old collected above rkm 120 in which were too decomposed for sexual maturity to be determined, the smallest of which was 1355 mm fl. The youngest confirmed male with stage 4 gonads was 11 years old with a fl of 1390mm. The only confirmed female was 21 years old and was 2005 mm fl.

Growth Curve Statistical analysis

Numerical convergence was achieved and parameter estimates with accompanying standard errors were calculated for all models fitted to the James River Atlantic sturgeon length-at-age data. Based on AIC_m and thus ΔAIC_m , the double von Bertalanffy model provided the best fit ($L_\infty=2241$ mm SE=271.9, $k_1=0.054$ SE=0.009, $k_2=0.097$ SE=0.033, $t_1=-2.85$ SE=0.23, $t_2=1.09$ SE=1.36), while the other four remaining models received considerably less empirical support (Table 1). A plot of residuals from the double von Bertalanffy model showed no apparent bias (mean of residuals = 2.9×10^{-3} , constant variance across age-classes), and the parameters were estimated precisely with the exception of t_2 . The estimate of k_2 was larger than the estimate k_1 .

Visual examination of the raw data showed an increasing rate trend in length-at-age between ages 5-10. The fitted double von Bertalanffy model captured this increase and is likely

why the double von Bertalanffy model fit the data better than other growth functions considered (Figure 3). The estimated pivotal age, t_p , was 6.03 years, which coincided with the approximate age that Atlantic sturgeon increasingly utilize oceanic habitats.

Discussion

The CV, 1.8%, was low compared to other sturgeon studies: 4.8% for Stevenson and Secor (1999; Atlantic sturgeon) and 7.8% for Rien and Beamesderfer (1994; white sturgeon, *A. transmontanus*). When an age discrepancy occurred reader 1 gave a higher age estimate (Figure 2). Even though a bias occurred in age determination, the low CV and average age discrepancy adds confidence to age estimations.

The youngest confirmed sexually mature male was 11 years old and had a fl of 1390 mm. The youngest freshwater samples were 10 years of age (n=3) and ranged in size from 1355-1458 mm fl. All three samples were too decomposed for sexual maturity to be determined. However, due to sample location we infer that these fish were likely mature. Therefore, James River male Atlantic sturgeon may mature as early as 10 years of age. Atlantic sturgeon maturation varies with age with age of maturity decreasing with lower latitudes (Smith 1985). Our age of male maturity results are between the estimations of 8 years for South Carolina and 11-20 years for the Hudson River (Dovel 1979; Smith 1985). The only confirmed female from this study was 21 years old with a fl of 2005 mm, however, only one specimen precludes reliable determination of female age-at-maturity. Female Atlantic sturgeon grow larger than males (Stevenson and Secor 1999) and the high male to female sex ratio in our samples likely lowered the right portion of the growth curve and the estimated L_{∞} . However; the one confirmed female in the study was only 9 cm larger than what the curve predicted.

Surprisingly, the growth function developed by Porch et al. (2002) had the second largest AIC_m value, despite the fact that it allows for the growth rate in proportion to length to vary gradually with age rather than no variation (as with the standard von Bertalanffy function) or change abruptly at a pivotal age (as with the double von Bertalanffy model). The higher estimate for k_2 over k_1 is unusual since the rate at which a fish approaches its maximum length generally decreases with age. However, the oldest sturgeon in the present study had an estimated age of 25 years, which is well below the historical estimated maximum age of 60 year (Magnin 1964).

Historical records document Atlantic sturgeon growing up to 4.5 m total length (Scott and Crossman 1973), almost three times greater than any samples in this study. The current James River population structure has changed from the historical population (Balazik et al. 2010). The present data set reflects the typical truncated age structure of a critically depleted population. Since the oldest sample was estimated to be 25 years, large degrees of age estimation error are not likely in this study as compared to sturgeon studies with 40+ to 100+ year old samples (Rien and Beamesderfer 1994; Stevenson and Secor 1999; Bruch et al. 2009).

Sampling effort for 6-9 year old fish was comparable to that for other age classes, yet few animals in this age range were collected. Tracking data in the James River indicates that Atlantic sturgeon in the 6-9 years of age size class are not utilizing the river as much as younger or older size class fish (Virginia Sturgeon Partnership, unpub. data). The perceived decreased presence of fish in this age/size range may indicate disproportionately higher utilization of oceanic habitats when compared to habitat utilization of other age classes. An increase in the growth coefficient around 6 years of age could be indicative of faster growth once in the ocean, possibly due to different prey resources. Larger sample sizes in the 6-9 years of age range are necessary to confirm patterns of ontogenetic habitat shifts during the first decade of life.

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List of Tables

Table 1. Residual sum-of-squares (RSS), Akaike's Information Criterion (AIC) and ΔAIC for the five growth models fitted to Atlantic sturgeon length-at-age data from the James River, VA. The double von Bertalanffy had the lowest AIC value indicating it is the best fitting model.

| Model | RSS_m | Number of parameters | AIC_m | ΔAIC_m |
|------------------------|---------|----------------------|---------|----------------|
| Double von Bertalanffy | 1.89 | 6 | -415.9 | 0.0 |
| Gompertz | 1.98 | 4 | -410.1 | 5.8 |
| Richards | 1.98 | 5 | -408.1 | 7.8 |
| Porch | 1.98 | 6 | -406.1 | 9.7 |
| von Bertalanffy | 2.02 | 4 | -405.3 | 10.6 |

List of Figures

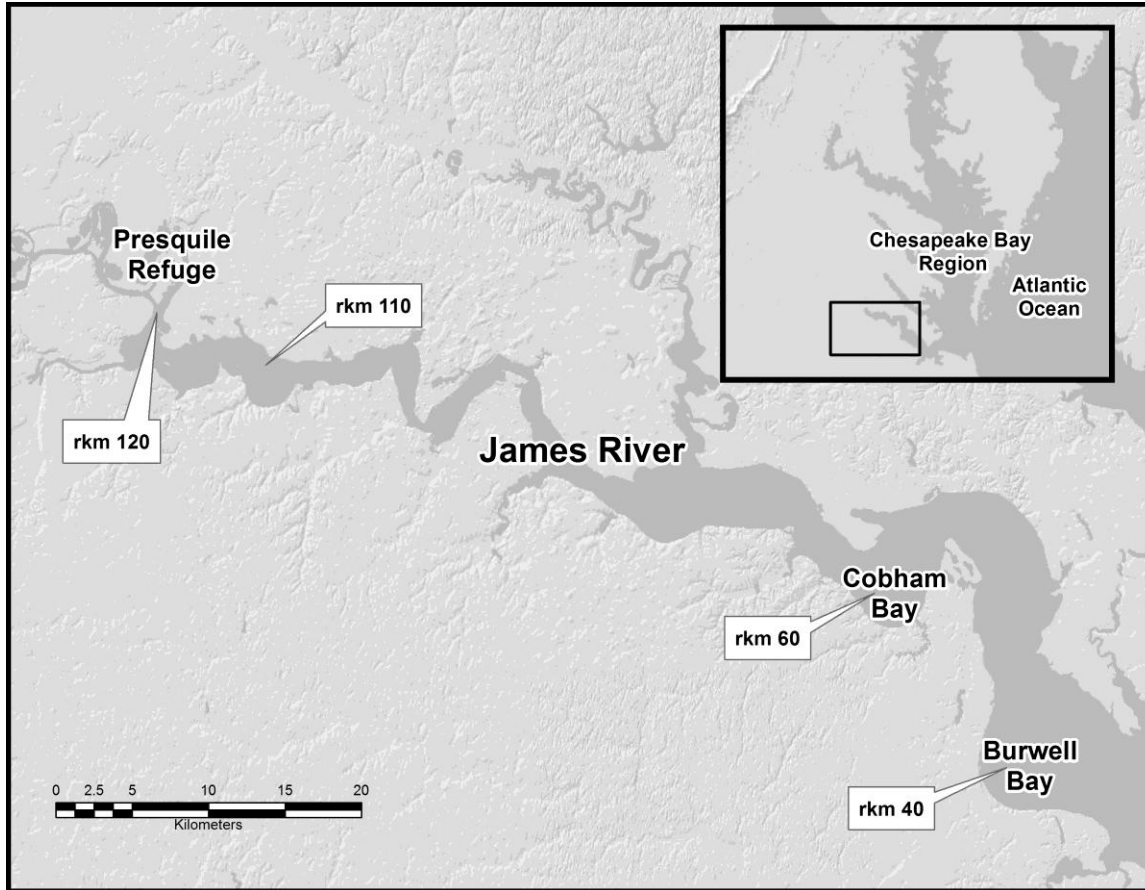


Figure 1. Map showing locations of James River Atlantic sturgeon sampling areas. The Burwell Bay (rkm 40) and Cobham Bay (rkm 60) areas are where the 202 juvenile/subadult Atlantic sturgeon were collected between 1997 and 2006. All samples from sexually mature fish (n=30) were vessel strike mortalities collected at or above Presquile National Wildlife Refuge (rkm 120) in August and September of 2006 through 2011 during putative spawning runs. The rkm 110 location signifies where the 135 mm young-of-the-year Atlantic sturgeon was caught in 2004 which verified spawning in the James River.

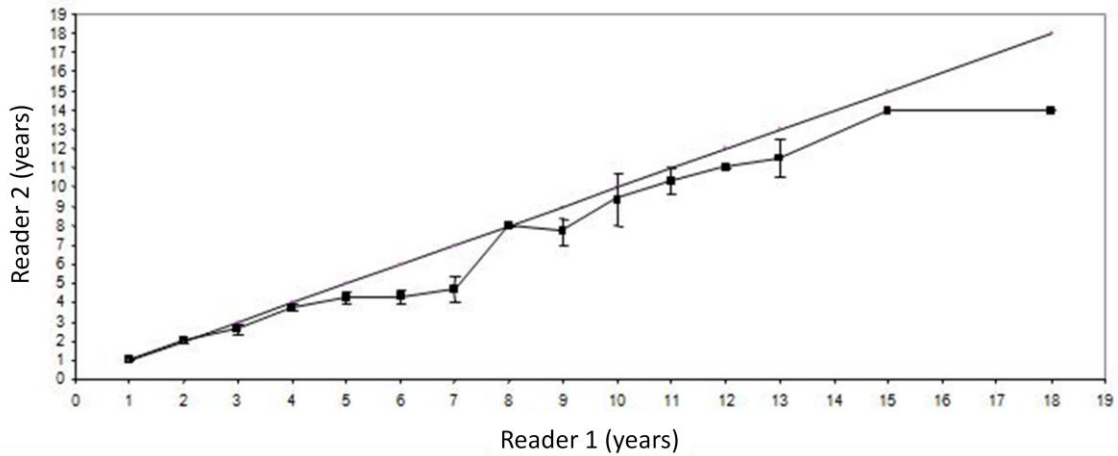


Figure 2. Age-bias plot visually displaying bias between reader 1 and supporting reader (reader 2) using James River Atlantic sturgeon pectoral fin spines for age estimation. The straight 1:1 background line indicates what one would expect if both readers had identical age estimates. The bias lines and points are consistently lower than the 1:1 line for older fish indicating that reader 1 consistently gave a higher age estimate compared to the supporting reader (reader 2). The error bars on the points indicate 95% confidence intervals of the mean age of reader 2 for all samples estimated to be a specific age by reader 1.

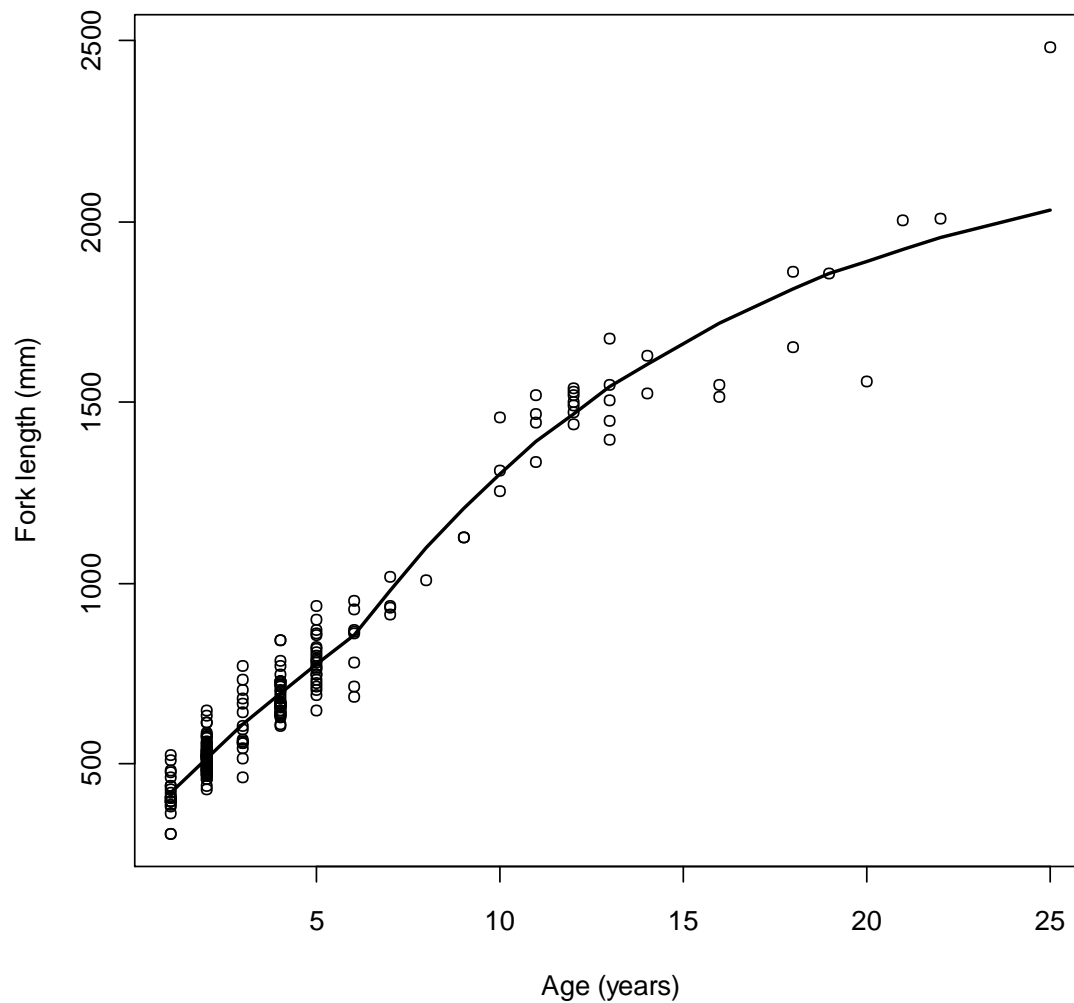


Figure 3. Observed length-at-age data and the fitted double von Bertalanffy model for Atlantic sturgeon in the James River, VA. The pivotal age, denoted t_p , was estimated to be 6.03 years. The bias correction factor based on the standard error of the estimate (SEE = 0.093) was incorporated into the back transformed model prediction (Sprugel 1983).

Chapter II

The potential for vessel interactions with adult Atlantic sturgeon in the James River, Virginia, USA.

Abstract

In 2012 all Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* populations were listed as either threatened or endangered under the Federal Endangered Species Act. During the period 2007-2010, researchers documented 31 adult Atlantic sturgeon carcasses in the tidal freshwater portion of the James River, VA. Twenty-six of the carcasses had gashes from vessel propellers, and the remaining five were too decomposed to determine cause of death. The types of vessels responsible for these mortalities were not explicitly demonstrated. Most (84%) of the carcasses were found in a relatively narrow reach of the river that was modified to increase shipping efficiency. To explore the number being hit and the horizontal and depth distribution of Atlantic sturgeon in relation to vessel draft, we conducted telemetry experiments on three living and six dead specimens. While staging, holding in an area from hours to days with minimal upstream or downstream movements, adult male Atlantic sturgeon spend most of the time (62%) within 1 m of the river bottom. Assuming behavior is not modified by vessel noise, adult male Atlantic sturgeon in the James River would rarely encounter small recreational boats or tugboats with shallow drafts suggesting mortalities are caused by deep-draft ocean-cargo ships. Dead specimens (n = 6) drifted with the current for several hours to almost 4 d before beaching at distances ranging from 0.5 to over 50 river kilometers from the point of release. We estimate that current monitoring in the James River documents less than one-third of vessel strike mortalities. A better understanding of Atlantic sturgeon behavior in the presence of vessels will aid the restoration progress of this federally-endangered species.

Introduction

The Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* is an anadromous fish that was abundant along the Atlantic slope of the USA until the early 20th century (Smith 1985; Bain 1997; Secor et al. 1997; Atlantic Sturgeon Status Review Team 2007). Due to unsustainable harvest, habitat loss, pollution, and dam construction, Atlantic sturgeon populations are severely depleted in the USA (Boreman 1997; Smith and Clugston 1997). During peak harvest at the end of the 19th century, the Chesapeake Bay was the second largest producer of Atlantic sturgeon products in the USA (Secor 2002). By the end of the 20th century the Chesapeake Bay Atlantic sturgeon population was considered functionally extirpated (Secor 1996; Speir and O'Connell 1996). Because of high spawning fidelity, a genetically distinct population segment (DPS) has developed in the Chesapeake Bay (King et al. 2001), and the Chesapeake Bay DPS was listed as endangered under the Federal Endangered Species Act in 2012.

The James River is one of two tributaries in the Chesapeake Bay with verified spawning success of Atlantic sturgeon over the past decade (Atlantic Sturgeon Status Review Team 2007; H. Brooks, Virginia Institute of Marine Science, unpublished data). Between 2007 and 2011, over 130 adult Atlantic sturgeon were collected in the tidal freshwater portion of the James River above river kilometer (rkm) 108.

Vessel interactions may have serious consequences hindering recovery of endangered aquatic species, including the Florida manatee *Trichechus manatus* (Laist and Shaw 2006) and various sea turtles families *Cheloniidae* and *Dermochelyidae*, (Chaloupka et al. 2008). Vessel interactions with sturgeon species have been documented in the Mississippi drainage (Gutreuter 2003, shovelnose sturgeon *Scaphirhynchus platyrhynchus*) and the Delaware River (Brown and Murphy 2010, Atlantic sturgeon). Annually since 2007, adult Atlantic sturgeon carcasses have

been identified during the fall (August 29-November 8) within the riparian zone of the tidal freshwater James River, VA (rkm 102-126). Twenty-six of 31 recovered carcasses were dismembered or had gashes that resembled vessel propeller strikes (Figure 1); the remaining five were too decomposed to determine cause of death. No Atlantic sturgeon carcasses without propeller strikes have been recovered in the James River suggesting the propeller injuries are the likely cause of death. In our observations gash marks are only found on the dorsal aspect except when the cut extends all the way through the fish. Fish must have had a normal erect posture when struck and were likely alive at the time because dead Atlantic sturgeon float ventral side up. Therefore, gashes unlikely occurred post-mortem. Propeller marks are also lacking on the ventral side of Atlantic sturgeon in the Delaware River (Matthew Fisher, Delaware Department of Natural Resources and Environmental Control, personal communication). On October 10, 2009 within 10 minutes after an ocean-cargo ship passed by a video was taken of an Atlantic sturgeon actively swimming at the surface with most of its head and tail dismembered. Although most carcasses are found by researchers (Virginia Commonwealth University and James River Association), there is no designated project to monitor for Atlantic sturgeon carcasses in the James River. Several other Atlantic sturgeon carcasses were reported by recreational boaters during the study period, but most were not confirmed and were not included in this analysis.

The threat of vessel strikes in riverine habitats could compromise Atlantic sturgeon recovery efforts by removing spawning members from the population (Boreman 1997; Brown and Murphy 2010). Vessel interaction is listed as a contributing factor for the species' decline (Atlantic Sturgeon Status Review Team 2007), and the actual number of vessel strike mortalities in the James River is unknown. The absence of population-size information makes it difficult to ascertain the proportion of spawning Atlantic sturgeon killed in the James River due to vessel

strikes. The vessel types striking adult Atlantic sturgeon and entrainment locations in the James River are also unknown. The objectives of this study were to: 1) determine amount of time adult Atlantic sturgeon spend at depths corresponding to various vessel propeller drafts, 2) identify areas where interactions are most likely to occur and 3) estimate the proportion of undetected vessel strike mortalities.

Study Area

The James River is the southern-most major tributary of the Chesapeake Bay (Figure 2). The freshwater tidal portion of the river extends up to Richmond at rkm 155. River width varies between 0.7 and 7.1 km up to rkm 120 and then narrows (range: 0.1 to 0.4 km). The federal navigation channel maintained by the U.S. Army Corps of Engineers runs from the river mouth to rkm 150. The channel is maintained to a minimum depth of 7.6 m and minimum width of 91.4 m. The Port of Richmond, located at rkm 145, is the major destination for deep draft vessel traffic upstream of where the river narrows at rkm 120. The area upstream of rkm 120 also accommodates tugboat and recreational boat traffic.

In 1934, the James River channel was modified by a constructed channel through an oxbow at rkm 120 to improve shipping efficiency. The smallest river width at this location is about 125 m. From 2007 to 2011 over 130 live adult Atlantic sturgeon have been caught and released within 2 rkm downstream of the modified river channel. This area seems to be an aggregation spot for adult Atlantic sturgeon during putative fall spawning periods. Most adult Atlantic sturgeon carcasses (84%) were found within 4 rkm of where the river narrows at rkm 120 (Figure 2). Because of the large number of live adult Atlantic sturgeon collected and high percentage of carcasses found in this area, we hypothesize this narrow cut through is a location where Atlantic sturgeon are being struck by vessel propellers. During the study period an ocean-

cargo ship completed one round trip to the Port of Richmond weekly. Tugboat and recreational boat traffic was common.

Methods

Live fish tracking

During fall 2008, three living, adult, male Atlantic sturgeon (spermiating) were collected by gill nets within 3 rkm of rkm 120 (Figure 2) and implanted (Kahn and Mohead 2010) with Vemco[®] V16 (estimated 1,630-d tag life) passive acoustic transmitters and Vemco[®] V13 ultrasonic transmitters (estimated 30-d tag life) equipped with a depth sensor (50-m range, 0.22-m resolution, $\pm 0.5\%$ accuracy) set at 0.75-s bursts. No female Atlantic sturgeon were tagged due to rarity of collection. Range tests were conducted on the tracking tags by anchoring the boat in a fixed position and anchoring tags at 10-m intervals between 10 m and 200 m from the boat. Tags were tracked using both omni-directional and directional hydrophones. To minimize effects of possible handling on fish behavior, data from the first 24 h of each track were excluded from the analysis. Individuals traveled a minimum of 2.7 km within the first 24 h and were therefore active post-surgery. After the initial 24-h period fish A was tracked for 33 h over the following 60 h, fish B was tracked for 55 h over the following 106 h, and fish C was tracked for 83 h over the following 172 h. Each fish was tracked by boat during day and night. Tracks were edited for accuracy, and spatial analysis was conducted using ArcMap (ESRI ArcGIS[®] version 10).

Vemco[®] ultrasonic tag transmissions may be received at a distance up to 1.5 km (Vemco[®] support), which complicates location determination. We used the global positioning system points of the tracking boat's location for analysis. To best approximate the position of the fish being tracked, the strongest signal from the first 30-s and last 30-s period of each minute

was used to infer the position of each fish. Because the channel width is a minimum of 91.4 m we only used pings determined to be emitted within 100 m of the boat. River bathymetry was determined using the National Oceanic and Atmospheric Administration's approach harbor soundings point shapefile.

Three major vessel types frequent the tidal freshwater James River: ocean-cargo ships, tugboats, and small recreational craft. The three vessel types have dissimilar drafts: small recreational craft (≤ 1.0 m), tugboat (≤ 2.3 m), and ocean-cargo ships (≤ 7.3 m when fully loaded), and therefore have propellers at different depths within the water column (U.S. Army Corps of Engineers, Norfolk District). Fish depths and locations were analyzed to determine time spent within each vessel type's draft depth. Bathymetry data were also used to determine water column preferences and time budgeting (100% means fish is at the surface, and 0% means fish is at the bottom) of tracked fish.

Carcass tracking

Between September 2 and October 8, 2009, six adult Atlantic sturgeon carcasses were implanted with long range (greater than 1.6 km) Advanced Telemetry Systems radio tags to assess mortality drift. Three of the carcasses were fresh mortalities with large gashes and the carcasses were positively buoyant. Two previously frozen carcasses were placed in a holding pen in the river and deployed after becoming positively buoyant. A third frozen carcass was allowed to thaw and released when internal body temperature was equal to water temperature; it sank to the bottom during initial release. Carcasses were released around rkm 120 during incoming or outgoing tides. Carcasses were re-deployed multiple times until too decomposed to handle, and 17 drifts were conducted. Carcasses were considered deposited after they remained in the same spot over a complete tide cycle. River discharge was monitored at rkm 110 to note if any

abnormal discharge events occurred during the carcass tracking time period that might affect deposit locations.

Results

Live fish tracking

The three live fish were tracked over a 55-rkm stretch of the James River, rkm 76 to 131, over an 8-d period (Figure 3). Atlantic sturgeon were present in the navigation channel 69% of the time while being tracked (Table 1), and there was no noticeable pattern to when or where they left the channel. On average fish spent 51% of the time while being tracked at depths coincident with the deep-draft ocean-cargo ship draft and were rarely at depths utilized by tugboats and small recreational crafts (Table 1, Figure 4). Above rkm 120 fish were within the draft of ocean-cargo ships 93% of the time. Fish A and B moved slowly back and forth with and against tidal flow and remained within 2 m of the bottom about 80% of the time (Figure 4). When downstream of rkm 120, fish B spent 20 h (19% of total h tracked) in deep holes out of all propeller ranges. While making a 7-h, 21-rkm downstream movement, fish C was within the navigation channel and ocean-cargo ship draft 72% of the time. Fish C did not remain consistently on the bottom but maintained a depth of roughly 5 m below the surface, even in water greater than 11 m deep (Figure 4). Cumulative histograms (Figure 4) indicate that fish A and B spent half their time within 1 m of the bottom and fish C within 4 m of the bottom, fish C remained higher in the water column than fish A and B, which were relatively stationary while being tracked.

Carcass tracking

No major discharge events that might influence carcasses deposit location occurred during the tracking period. Sixteen of the 17 carcasses set adrift were located. The carcass that

initially sank stayed stationary on the bottom for almost 3 d until it became positively buoyant and started to move. Similarly, the two fish placed in a pen became positively buoyant within 3 d. While being tracked, positively buoyant carcasses remained at the surface ventral side up until stranded. Drift distances ranged from 0.5-52.6 rkm with an average of 10.8 (SD, 13.8 rkm) from the release point (Figure 2), and the average drift time and SD was 40 ± 24 h (range 4-93 h). The drifting carcasses were observed moving back and forth with the tide several times before depositing. At least twice a carcass was pushed onto the beach by the incoming tide but drifted off during the next outgoing tide. Carcass deposition was witnessed six times and occurred within 1 h of tide change in four cases. Likely only 5 (31%) of the carcasses would have been found using current monitoring techniques. The remaining 11 carcasses (69%) were deposited in areas rarely frequented by researchers. Also, several carcasses were covered by vegetation/driftwood and were obscured when viewed from the water; however, this factor was not used to determine whether the carcasses would be considered found or not.

Discussion

The Atlantic Sturgeon Status Review Team (2007) stated that rivers with narrow channels and large vessel traffic have high incidences of vessel strikes on adult Atlantic sturgeon. Brown and Murphy (2010) described the number of mortalities and the potential impact on the Atlantic sturgeon population due to vessel interactions in the Delaware River. To our knowledge, this study is the first published study to use active telemetry to explore vessel interactions with adult Atlantic sturgeon. River morphology constrains adult Atlantic sturgeon to the navigation channel while inhabiting the tidal freshwater portion of the James River above rkm 120 during the fall season. Large ocean-cargo ships are the main vessel type whose draft intersects with adult male Atlantic sturgeon during their fall river residence.

Ryder (1890) noted spawning Delaware River Atlantic sturgeon tended to stay on the bottom except when moving rapidly. Fish A and B moved minimally while being tracked and occupied a position within 1 m of the river bottom, similar to white sturgeon *A. transmontanus* in the Kootenai River, British Columbia (Paragamian and Duehr 2005). Most areas of the James River above rkm 120 are maintained at the navigable channel depth minimum of 7.6 m; therefore, Atlantic sturgeon utilizing the tidal river above rkm120 are consistently at ocean-cargo ship propeller depth. The federal navigation channel occupies a 25-rkm stretch where the river's narrow width and channel depth form an area in which adult male Atlantic sturgeon have an increased risk of injury and mortality from ocean-cargo ships compared to downstream areas with deep water refuges. While being tracked during a 7-h downstream movement of 21 rkm, fish C maintained a depth of about 5 m below the surface even in water greater than 11 m deep (Figure 4). Passive data from fish that returned twice in subsequent years indicated fish maintained similar depths as the active tracking data. On one occasion a fish was actively tracked during an encounter with an ocean-cargo ship while in the cut-through above rkm 120. Because the tracking boat had evacuated the immediate vicinity, fish behavior while the ocean-cargo ship passed over is unknown. However, upon return (moments after ship moved through the cut-through) we noted that the fish had maintained about the same place before and after the ocean-cargo ship passed over. This observation was within the first 24 h after tag implantation.

Atlantic sturgeon carcasses drift much further than we hypothesized based on recovery distribution. Only 31% of the drift mortalities deposited in areas frequented by researchers or the riverkeeper. The other 69% deposited in areas not monitored during other projects. If 31% of vessel strike mortalities from 2007 to 2010 were found, extrapolation suggests 80 adult Atlantic sturgeon were killed over that time. Only 21% of the constricted reach of the James River is

monitored during other research projects in the area, leaving the other 79% mostly uninvestigated. Therefore the estimated 80 mortalities is extremely conservative. Gutreuter et al. (2003) estimated 0.53 shovelnose sturgeon are entrained per km traveled by towboats in some areas of the Mississippi and Illinois rivers. The Gutreuter et al. (2003) study applies to commercial ships in narrow channels, similar to the James River/ocean-cargo ship situation.

The drift movement of Atlantic sturgeon carcasses complicates determination of vessel strike location. Carcasses can drift over 52 rkm in the James River, requiring a large area to be surveyed. We frequently found mortalities on bare beaches. However, many of the carcasses deposited in areas covered by vegetation and drift wood, which makes it difficult to locate dead Atlantic sturgeon by boat from the water.

Vessel traffic is expected to continue in the tidal freshwater portion of the James River. Deeper channel dredging is unlikely due to hard-bottom substrate. Therefore, vessel strikes will likely continue and may hinder species recovery. Future work is needed to better understand Atlantic sturgeon behavior in the presence of vessels in order to reduce encounters. Also, a dedicated monitoring program would allow a more accurate enumeration of adult Atlantic sturgeon mortalities during their riverine residence.

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Sarah Cameron (U.S. Army Corps of Engineers , Norfolk District) for boat draft information; and Jed Brown (U.S. Virgin Islands Division of Fish and Wildlife, St. Croix) and Matthew Fisher (DNREC-Division of Fish and Wildlife) for information on Delaware River Atlantic sturgeon vessel strikes. We thank the PADI Foundation for partial financial support. This is VCU Rice Center Contribution number 25.

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List of Tables

Table 1. Percentage of time actively tracked adult male Atlantic sturgeon inhabited the same draft of various vessel types. The table is separated by total time and time within the federal navigation channel. The final column shows what percentage of the water column (100% fish is at the surface, 0% fish is at the bottom) the tracked Atlantic sturgeon inhabited.

| Fish ID | Time in navigation channel | Small recreational (≤ 1.0 m) | Tugboat (≤ 2.3 m) | Ocean-cargo (≤ 7.3 m) | Below all draft depths | Average area of water column |
|---------------------|----------------------------|------------------------------------|-------------------------|-----------------------------|------------------------|------------------------------|
| Total time | | | | | | |
| A | 67 | 0 | 0 | 49 | 50 | 8 |
| B | 83 | 0 | 0 | 21 | 78 | 9 |
| C | 56 | 0 | <1 | 84 | 15 | 32 |
| Time within channel | | | | | | |
| A | 100 | 0 | 0 | 58 | 42 | 7 |
| B | 100 | 0 | 0 | 22 | 78 | 9 |
| C | 100 | 0 | <1 | 87 | 12 | 36 |

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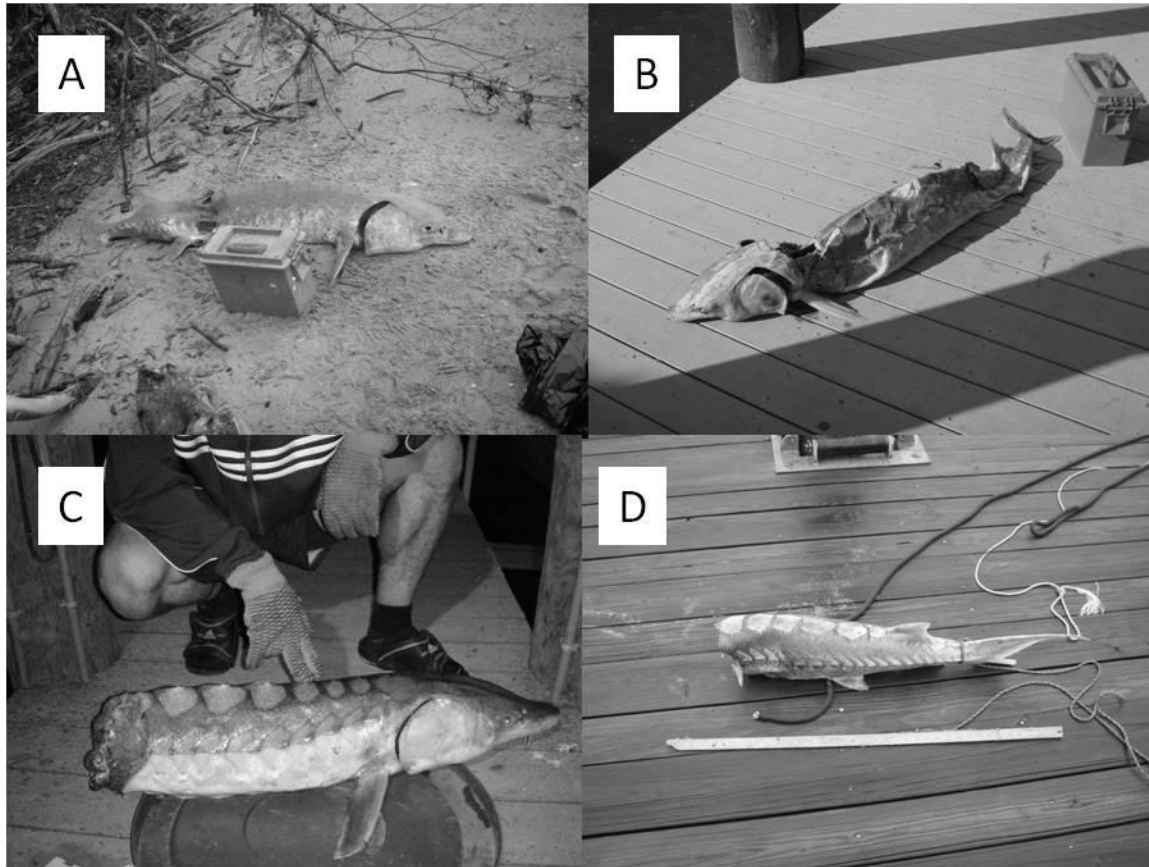


Figure 1. Examples of Atlantic sturgeon hit by vessel propellers in the James River, VA, during the fall. Most (84%) were found above rkm 115 and all were estimated to be over 148 cm fork length (FL). Picture A is of a post-spawn female (183 cm FL) and picture B is a male (162 cm FL), both with large gashes on the dorsal side. Fish C and D were males dismembered, and both were estimated to be 150 cm FL.

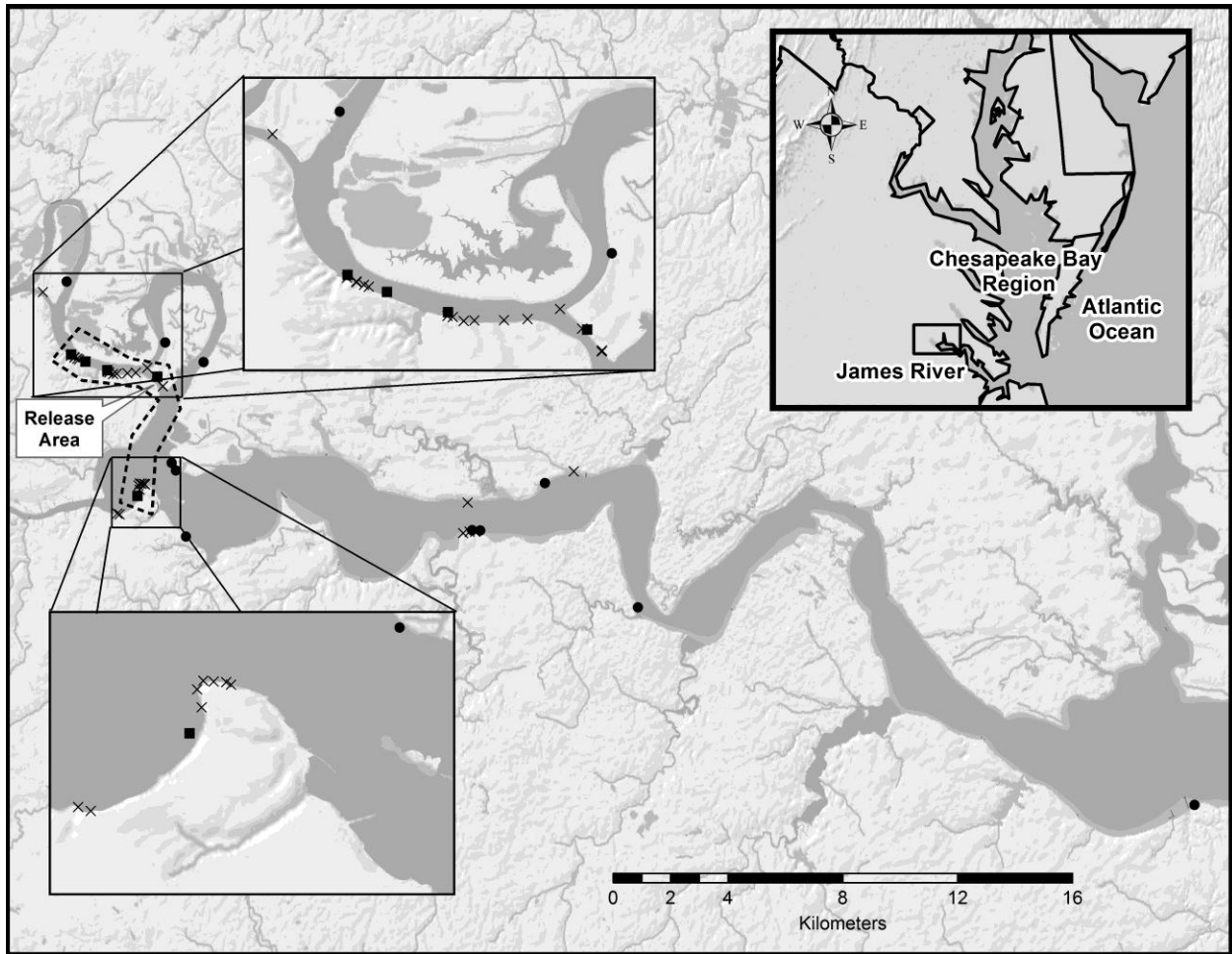


Figure 2. The James River, VA, between river kilometer 70 and 127 showing locations where 31 serendipitously discovered adult Atlantic sturgeon (X) carcasses were found between 2007 and 2010. Two areas of high carcass densities are enlarged. The dashed box on the left side of the map indicates the area researchers consistently conduct research during the fall. The black squares (▪, n = 5, likely would be documented) and black circles (●, n = 11, likely would not be documented) show deposit sites of 16 Atlantic sturgeon carcasses released by researchers at river kilometer 120.

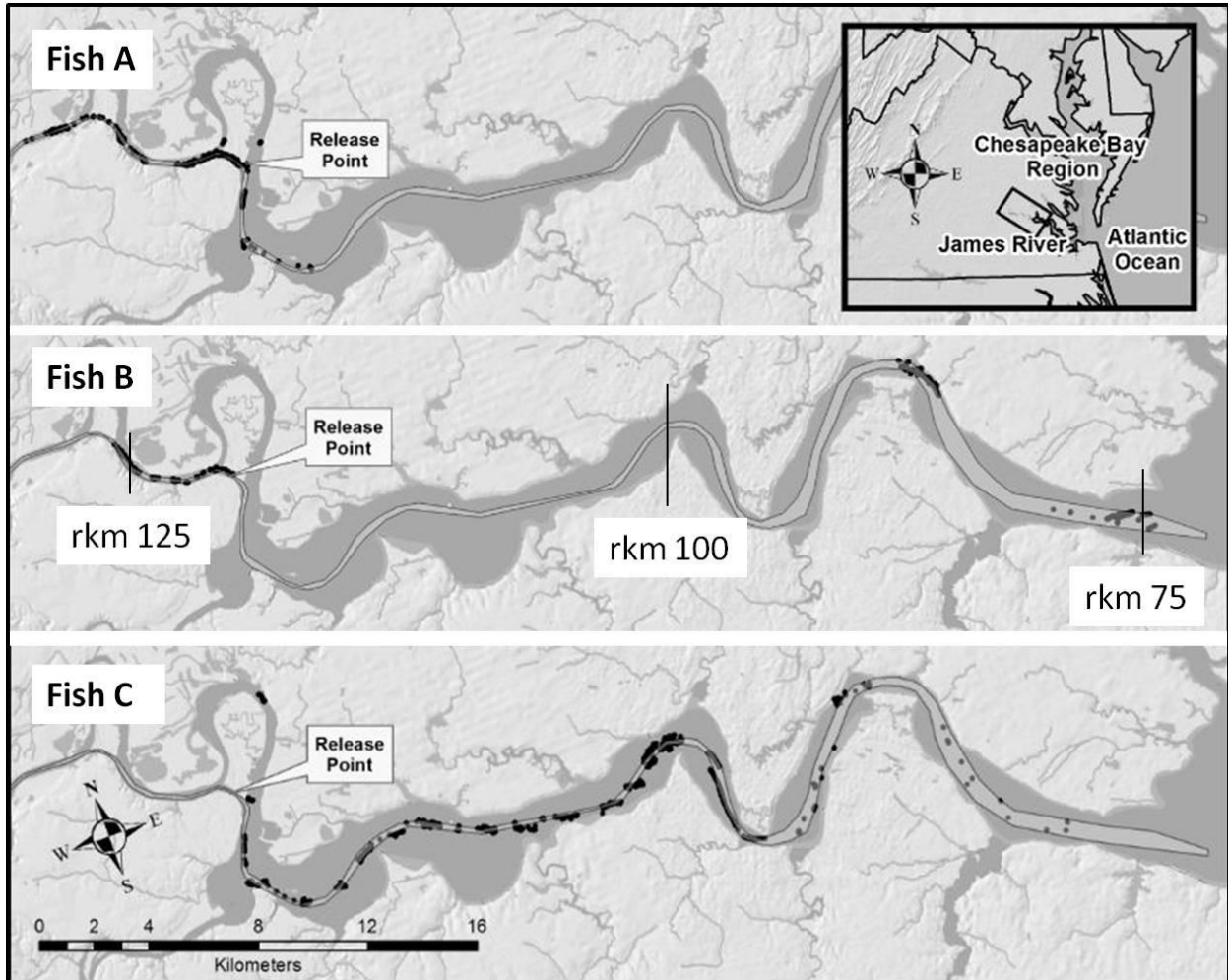


Figure 3. Map showing the James River, VA (river kilometer 72 to 134), river channel and active tracks of three adult male Atlantic sturgeon. Fish were released at rkm 120 where the river width narrows by over 60%. Note that tracks are mostly confined within the channel. Both fish A and B did not make any long directional movements while being tracked and mostly stayed stationary or slowly moved back and forth in a small area. However fish C made a 21-rkm downstream movement that took 7 h and maintained a water column depth of about 5 m.

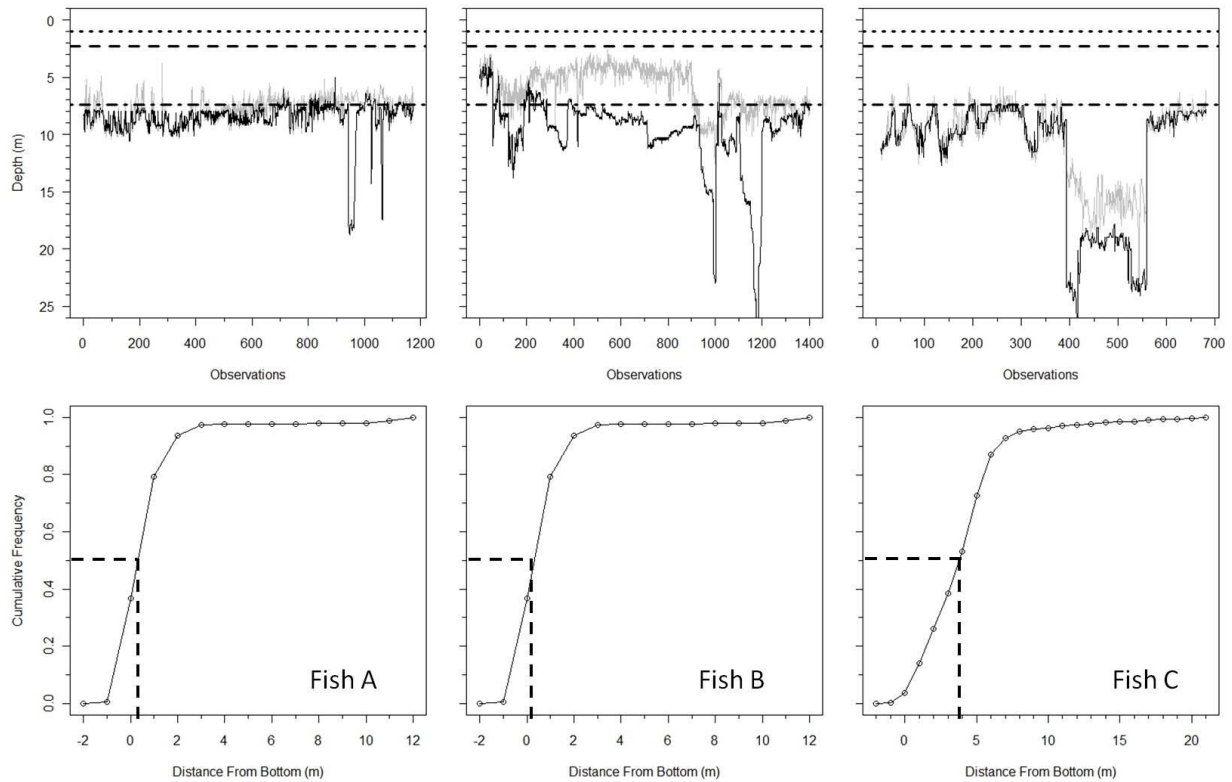


Figure 4. The top three plots show fish depth (grey line) and river bottom depth (black line) of the three tracked adult male Atlantic sturgeon. The three dashed black horizontal lines show various vessel draft depths: $1 \text{ m} \leq$ small recreational craft (.....), $2.3 \text{ m} \leq$ tugboat (-----), and ocean-cargo vessel (-.-.-.). The bottom three graphs are cumulative frequency plots showing distance-from-bottom frequencies for each fish. Fish A and B were tracked while somewhat stationary and stayed close to the bottom at various channel depths. Fish C was tracked during a 21-rkm movement taking 7 h and maintained a depth of about 5 m during the move even when in much deeper water.

Chapter III

Empirical evidence of fall spawning by Atlantic sturgeon in the James River, Virginia, USA.

Abstract

Due to overfishing and habitat alteration, the anadromous Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) is severely depleted across its historic range. The James River and York Rivers, Virginia are the two rivers comprising the Chesapeake Bay distinct population segment where Atlantic sturgeon reproduction has been confirmed. It is widely recognized that Atlantic sturgeon spawn in the spring throughout their range; however, there is debate over whether they also spawn in the fall. To determine if Atlantic sturgeon spawn in the fall, independent of the spring spawn, large mesh gill netting in the freshwater portion of the James River (above rkm 108) was conducted in the spring (April-June) and fall (August-October) for three years (2009-2011) resulting in the capture of 125 adult Atlantic sturgeon (three were recaptures) during the fall sampling, but none were captured during the spring. Field examination for sex and stage of maturity identified 106 mature males, and one post-spawned female. Sex was not determined for four fish, and due to time constraints 11 were not examined. Forty mature males were externally tagged with Vemco[®] ultrasonic passives tags and movements were monitored with a Vemco[®]

VR2W passive receiver array. Collection and tracking data showed that mature Atlantic sturgeon aggregate in the freshwater portion of the James River during the fall season, entering during August and out migrating by end of November. No tagged fish were detected in the freshwater area of the river during the subsequent spring months. Though James River Atlantic sturgeon may spawn in the spring, we suggest there is strong evidence for an independent fall spawn which should be considered in future management and recovery actions.

Introduction

Due to overfishing and habitat alteration the anadromous Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) is severely depleted along its range from Labrador, Canada to Florida, United States (Hildebrand and Schroeder 1928; Smith 1985; Bain 1997; Boreman 1997). The National Oceanic and Atmospheric Administration (NOAA) recognizes five genetically distinct population segments (DPS) along the east coast of the United States (King et al. 2001; Atlantic Sturgeon Status Review Team 2007), and in 2012 all were listed as either threatened or endangered under the federal Endangered Species Act (Federal Register/Vol. 77, No. 24). The Chesapeake Bay DPS was listed as endangered, however, in the Atlantic Sturgeon Status Review (2007) spawning was assumed to occur only in April-May. It would be important for future recovery efforts of this DPS to know if there are two spawning populations (spring and fall), to better manage river activities that might interfere with spawning adults, and improve population estimates. Activities currently regulated include the timing of dredging in the James River, to accommodate spring spawning of anadromous fishes. Perhaps dredging activities should also be regulated during the fall if spawning Atlantic sturgeon are identified.

The concept of both vernal (spring) and hiemal (fall) migrations of anadromous fishes was reviewed by Berg (1959) but no sturgeon species were identified as spawning in the fall. It was reported that fall run sturgeon (sturgeon that migrate to freshwater in the fall) typically overwinter and then spawn the following spring. Shubina et al. (1989) also indicated that stellate sturgeon (*A. stellatus*) in the Kura, Don and Danube Rivers have two pronounced seasons of spawning runs in the spring and fall, but the fall run sturgeon overwinter and spawn in the spring.

Since Berg's (1959) review sturgeon migrating and spawning upriver in the fall has been reported for several Eurasian and North American species. Vlasenko et al. (1989) reported that Persian sturgeon (*A. persicus*) “spawns in the rivers of the southern Caspian region from April through June and again in August and September”. Spawning of the Chinese sturgeon (*A. sinensis*) in the Yangtze River during October-November has been well documented with the capture of fertilized eggs (Wei et al. 2009). Tripp et al. (2009) reported that reproduction of Mississippi River shovelnose sturgeon (*Scaphirhynchus platyrhynchus*) could be protracted into the fall or bimodal (i.e., spring and fall peaks) when fall environmental conditions are similar to those that occur during the spring. They reported milting males and females with eggs in spawning condition during the fall, and collected an age-0 shovelnose (55 mm total length) during November that was probably spawned in September, based on its size and the estimated growth rate. Evidence of fall spawning has also been recently reported in the Gulf sturgeon (*A. o. desotoi*), a subspecies of the Atlantic sturgeon (Randal and Sulak 2012).

It is widely recognized that Atlantic sturgeon spawn in the spring (Smith 1985, Smith and Clugston 1997; Kynard and Horgan 2002), although evidence of fall spawning Atlantic sturgeon was reported in several cases. Worth (1904) in North Carolina and Smith et al. (1984) in South Carolina describe reduced runs, compared to the spring runs, of Atlantic sturgeon during the autumn months. Collins et al. (2000) observed movements of two sturgeon in the Edisto River, South Carolina, that were strongly indicative of a fall spawning migration, and captured a very recently spawned female at river kilometer (rkm) 56 in the Edisto River during the fall. A fall spawning season is also suspected to occur in the Altamaha River, Georgia (D. Peterson, University of Georgia, personal communication). In August of 2007 adult size Atlantic sturgeon were seen breaching between rkm 105 to the fall line at rkm 155 of the James River, and since

Ryder (1890) describes Atlantic sturgeon breaching coinciding with spring spawning runs, the collection efforts for adult Atlantic sturgeon during the fall season began in 2007. Organized sampling in the spring and fall was not started until 2009.

Our hypothesis is that James River Atlantic sturgeon spawn in the fall, and the objectives of this study, were: 1) capture mature adults during the fall and compare with spring captures; 2) determine sex and stage of maturity of individuals; 3) track movements of adults using ultrasonic tags; and 4) examine seasonal frequency of reported vessel strikes on sturgeon.

Methods

Adult Capture, Body Morphometrics, and Stage of Maturity

From 2009 through 2011 efforts to catch mature Atlantic sturgeon above rkm 108 of the James River were conducted in both the spring (Apr-June) and fall (Aug-Oct). NOAA Atlantic sturgeon collection and handling guidelines were followed (Mohead and Kahn 2010). Every year the same nets of various mesh sizes (25.4-35.6 cm stretch mesh) and heights (2.5-5.3 m deep) were used, and all nets had a length of 91.4 m. Sink gill nets were set parallel to the current in water depths ranging from 3.7 m to 11.6 m, as most Atlantic sturgeon utilize the lower portion of the water column during spawning runs (Ryder 1890). Sink gill nets were set and fished the same way during both seasons. Gill nets were allowed to soak night (when temperatures permitted) and day during the spring, but fall gill net sets were limited to daylight hours. Drift netting (30.5 cm stretch mesh, 4.8 m deep) was attempted in the spring of 2011. For this study we considered effort to be the total net-hours fished. Surface water temperatures ($\pm 1.0^{\circ}\text{C}$) were recorded every day at gillnet sampling locations.

Fork length (FL) and the largest girth (typically between the fourth and fifth dorsal scutes) were measured (+/- 1.0 cm) (Kahn and Mohead 2010), and fish were weighed (+/- 1.0 kg). Condition factor was determined by

$$\frac{\text{Weight}}{\text{Fork Length}^3} \times 100000$$

with weight in kg and FL in cm.

Sex was determined by our ability to manually express semen or eggs (Mohler 2004), but if no gametes were expressed, a 10 cm section of tygon tubing (6.4 mm outer diameter, 4 mm inner diameter) was gently placed into the vent and then angled slightly towards the left or right gonad, during insertion. All fish were externally (t-bar) and internally (passive integrated transponder, PIT) tagged (Damon-Randall 2010).

In 2009 and 2010 semen samples from three different fish were collected to confirm sperm motility. The area around the urogenital opening was dried to prevent collected semen from being activated prematurely by contact with water or urine. A 10 cm length of tygon tubing (6.4 mm outside diameter) was attached to a 60 mL syringe and inserted into the urogenital opening to extract semen. Semen samples, up to 25 mL per sturgeon, were placed into 50 mL sterile tubes (Dorsey et al. 2011). The tubes were bubble wrapped, placed in a cooler with ice packs, and transported to Virginia Commonwealth University (VCU). The semen was activated using distilled water in 2009 and James River water in 2010 within 6 hours of collection and viewed under a compound microscope (40× magnification). During 2011 semen was collected from 14 spermiating males captured during September and October. Semen samples were collected following the same protocol described above, however, sample tubes containing the semen were purged with oxygen and capped tightly and then placed in a ThermoSafe[®] insulated shipper (ThermoSafe Brands, Arlington Heights, Illinois) containing a frozen gel pack, which

was insulated with layers of paper to keep from direct contact with the sample tubes, for overnight shipment to the University of Maryland -Crane Aquaculture Facility (UMCAF).

On the day that a semen sample arrived at UMCAF, semen characteristics were determined including: the percent motile sperm, osmolality (mOsm/kg), pH, and sperm density (cells/mL of semen). A single aliquot was analyzed for each male. Spermatozoa were activated by adding 18 μ L of 20mM Tris-NaCl (80 mOsm/kg, pH 8.0) to 2 μ L of semen placed in a Makler[®] counting chamber (Sefi Medical Instruments, Haifa, Israel). Motility and sperm density were determined from digital recordings made from the activation subsample with a Magnavox[®] model ZC320MW8 digital recorder (Philips Electronics, Andover, Massachusetts) and a Hitachi[®] Model KP-D20BU high contrast color digital camera (Hitachi, Tokyo, Japan) attached to a Zeiss[®] Model D-7082 phase-contrast microscope (Carl Zeiss, Berlin, Germany) at 200 \times . Osmolality and pH were determined by means of a Westcor[®] Model 5400 vapor pressure osmometer (Westcor, Logan, Utah) and a Hach[®] Model Sension2 pH electrode (Hach, Loveland, Colorado), respectively.

Telemetry

Over the 3-year sampling period, forty mature males were externally tagged under our VCU Animal Use and Care Protocol # 20127, with ultrasonic tracking tags (Vemco[®] model V-13 or V-16, battery life 3+ years, Halifax, Nova Scotia, Canada). Tag range (1+ km) provided complete river width coverage in several areas of the river. Movements of the fish tagged with ultrasonic transmitters were determined using Vemco[®] model VR2W receivers previously deployed in a 30-receiver array monitored by the Virginia Atlantic Sturgeon Restoration Partnership.

Vessel Strikes

The Virginia Atlantic Sturgeon Restoration Partnership has recorded the number of sturgeon mortalities reported to have injuries associated with vessel strikes, such as deep lacerations on the head or body regions. These injuries seem to be from large ocean tankers ships that run consistently year round (See Chapter II).

Results

Adult Capture, Body Morphometrics, and Stage of Maturity

Sampling in 2009 totaled 240 net-hours in the spring and 513 net-hours in the fall. In 2010 sampling was increased to 1232 net-hours in the spring and 949 hours in the fall. Effort in 2011 totaled 1272 net-hours in the spring and 462 net-hours in the fall (Table 1). In 2011 drift nets were drifted for a total of 26 h with no collections or signs of Atlantic sturgeon interactions.

Over the duration of the study 122 different Atlantic sturgeon ranging from 127-203 cm FL were captured in the fall (August 5-October 9) but none were captured in the spring (Table 1). All fall collections were made between rkm 108 and 132 (Table 1). Water temperatures ranged from 19-30°C during the fall sampling period. Of the 122 captures, 106 were determined to be mature males because all released whitish colored semen from the vent, when manual stripping of the abdomen was performed. Males ranged in size from 127 to 181 cm FL, girth varied from 56 to 82 cm, weights were between 19 and 51 kg, and condition factors ranged from 0.53 to 1.03 (Table 1). Many (approximately 40%) males were observed releasing semen during gill net retrieval, and one male caught on October 2, 2009 was recaptured within 250 m of his original catch location on September 16, 2010 and was expressing semen on both occasions. The cursory examination of 2009 and 2010 semen samples verified that activated semen was motile from all six males, for approximately one minute. The more extensive examination of the

2011 samples showed variation in semen characteristics (Table 2). Sperm density varied from $1.2 \times 10^9/\text{mL}$ to $7.1 \times 10^9/\text{mL}$ and percent sperm motility ranged from 0% to 90%.

One individual had a concave abdomen, and did not release semen when hand-stripped. The vent was then checked with the catheter and three eggs were recovered indicating that it was a post-spawned female. The confirmed post-spawned female was caught September 9, 2011 with nine other males, all of which were expressing semen during gill net retrieval. The female had a FL of 170 cm, weighed 45 kg, girth of 76 cm, and condition factor of 0.92.

Four individuals were captured but sex was not verified as neither eggs nor semen were expressed or obtained when the vent was catheterized, and all were caught between September 9-17 across all three years. One of these fish, captured on September 9, 2009, had a concave abdomen similar to the post-spawn female from 2011. This fish had a FL of 186 cm, girth of 87 cm, weight of 58 kg, and a condition factor of 0.90. The three remaining fish (one captured twice) had firm abdomens and were both stripped and catheterized. They had an average FL of 198 cm (195-203), average weight of 89 kg (87-93), average girth of 109 cm (90-121), and average condition factor of 1.13 (1.09-1.19).

Telemetry

Ultrasonic tags were attached to five fish in 2009, 25 in 2010, and 10 in 2011 (Table 3). All tagged fish were male and departed the James River into the Chesapeake Bay by November 8 (Table 3). One fish (tagged in fall 2009) returned to the James River on August 15, 2010 and departed October 26, 2010, after having moved upstream of rkm 145 (Table 3). Eight of 30 individuals tagged in 2009-2010 returned to the James River in May and early June of 2011, but none of the fish went upstream of rkm 60, which is downstream of the salt wedge. All of these males left the James River within 22 days after arrival; however, four of them returned again in

August along with another eleven 2009-2010 tagged adults. These individuals moved upstream to at least rkm 133 and most went upstream of rkm 143, just downstream of the fall line, at rkm 155. All 16 fish that returned in later years left the James River by November 3 (Table 3). We found no evidence suggesting that mature fish overwintered in the river.

Vessel Strikes

Since 2007 in the James River there have been 34 adult sturgeon carcasses found between August 28 and October 19, while none have been found during the spring months (Virginia Atlantic Sturgeon Restoration Partnership, unpublished).

Discussion

There are several observations which support our hypothesis of a fall spawning season for Atlantic sturgeon in the James River: (1) the seasonal capture, and physiological stage of maturity of fish captured during the 3-year study, (2) the ultrasonic tagging and tracking data, and (3) the seasonal frequency of reported vessel strikes on mature fish. In addition, numerous fishers, guides and watermen report breaching adult sturgeon beginning in August and lasting for several months, but no observations of breaching have been documented during the spring.

One captured fish was identified as a female during the 3-year study, and the concave condition of the abdomen was consistent with female sturgeon that have spawned recently (Ryder 1890). In addition, post-ovulated eggs recovered from the urogenital opening were in an early degradation stage also suggested the fish had spawned within days (J. Van Eenennaam, University of California (Davis), personal observation). Further physiological support for fall spawning is provided by the nine spermiating males captured along with the female and a grand total of 106 different spermiating males captured during August-October. Randall and Sulak (2012) reported similar evidence for fall spawning of the closely related Gulf sturgeon, which

included multiple captures of sturgeon in September-November that were ripe or exhibited just-spawned characteristics.

Water temperatures varied throughout the sampling season, and while no gillnet mortalities occurred in early August, 2009 because of high water temperatures (30°C), we delayed our sampling efforts until water temperatures were less than 26°C. The water temperature was 25°C at the capture location of the confirmed female, and although this was above optimum for spawning, water temperatures are usually 1-2°C lower at the fall line, which is where spawning events are likely occurring. By mid-September water temperatures were typically around 20-23°C at our capture locations which is partially within optimum spawning conditions (20-21°C) for Atlantic sturgeon (Mohler, 2004).

Ripe males expressed semen with no or little effort from researchers, and analysis showed it was motile. Some semen samples had 80% sperm motility even after two days post-collection. The three samples with 0% sperm motility may have been contaminated with water or urine. Three of the four fish in which sex could not be determined had higher condition factors, weights, lengths, and girths compared to all the male fish collected throughout the study, and their average size was larger than the average mature female captured during the Hudson River spawning run (Van Eenennaam et al. 1996). The condition factors for these three fish were also higher than a confirmed female (condition factor of 1.08) recently caught by commercial watermen conducting a survey for a fisheries resource grant. This particular female was caught in the James River around rkm 27 on April 20, 2011, and had ovaries with 1.5-1.7 mm diameter pigmented oocytes (M. Balazik, Virginia Commonwealth University, unpublished data) which is smaller than fully mature eggs that are usually about 2.6 mm in diameter (Ryder 1890; Van Eenennaam et al. 1996; Mohler 2004). Thus, the large body size, girth, and condition factor

suggests the three fish could have all been mature females. However, there is also the possibility that some of them were vitellogenic females (1-2 years from spawning) or even large maturing males. Non-reproductive sturgeon have been captured during the spring migration into freshwater (Van Eenennaam et al. 1996; Sulak and Randall 2002; Webb and Erickson 2007) and it is hypothesized that they enter the river to feed or it is a possible learning behavior to locate specific spawning or habitat sites (Sulak and Randall 2002; Webb and Erickson 2007). The remaining non-sexed fish had a concave abdomen similar to the verified post-ovulatory female, and although no eggs were collected, the fish's morphology is consistent of a recently spawned female (Ryder 1890).

Adult fish that were tagged with ultrasonic transmitters above rkm 108 returned in later years to the freshwater portion of the river (upstream of rkm 133) in August and left the James River by late November. The seasonal absence of telemetry data in freshwater during winter and spring months for adult fish tagged in the fall suggests the fish are not holdovers from the spring. We found no adult Atlantic sturgeon tagged in the fall that overwintered in the James River suggesting fish that migrate upstream and out migrate in the fall.

The seasonal pattern of vessel strikes also supports the hypothesis for a fall spawning season under the premise that greater numbers of adult Atlantic sturgeon would be present in the river during a spawning migration. Boat strike mortalities on adult Atlantic sturgeon have also been reported during the spring spawning run on the Delaware River (Brown and Murphy 2010).

Additional evidence of fall spawning from another Chesapeake Bay DPS river is represented by the recent capture of four age-0 (FL13-15 cm) Atlantic sturgeon in the York River, December 2011- January 2012 (H. Brooks, Virginia Institute of Marine Science, personal communication). Using Delaware River Atlantic sturgeon age-0 growth data, the York River

age-0 fish were estimated to be 2.5 to 3.5 months post-hatch (M. Fisher, DNREC-Division of Fish and Wildlife, personal communication) suggesting they were hatched in approximately the same time frame (September) as the captured post-spawn female in the James River. Randall and Sulak (2012) reported a similar observation, with the capture of a 9.3 cm TL age-0 Gulf sturgeon on November 29, 2000 which would have come from a late September spawn.

Should sexually mature fish be captured in the future, during the spring or early summer and again in the fall, genetic analysis could be used to determine if the fall spawners are a distinct population. However; even if genetic differentiation is not found between spring and fall fish, our data reveals high usage of the freshwater portion of the James River from August-November by mature Atlantic sturgeon, a factor that could be important in future fishery management decisions. The Virginia Marine Resources Commission restricts dredging in the James River from March 15 through June 30 to accommodate spring spawning anadromous fish, and the US Army Corps of Engineers restricts dredging activity in the Savannah River, South Carolina, from March 16 through May 31 to prevent interference with Atlantic sturgeon transit. As such, these restrictions are inadequate for protection of vulnerable life stages of Atlantic sturgeon related to a fall spawning season. In addition, if there is a fall spawning run of James River Atlantic sturgeon then the existing population size estimates may be in error if consideration is only given to adult collections made in the spring of the year.

Although our results provide evidence that fall spawning of Atlantic sturgeon occurs in the James River, we recommend further verification studies that could include: deployment of egg collection mats, additional broodstock collection, sexing and staging maturity using ultrasound, gonad biopsy and/or plasma steroid analyses, continued acoustic tagging and

tracking of adults, genetic analyses of spring vs. fall-captured adults, and additional collection/length-frequency evaluation of age-0 individuals.

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List of Tables

Table 1. Seasonal sampling effort, dates and locations of capture, river water temperature, body size and condition factor for mature male Atlantic sturgeon collected during the fall 2009-2011.

Data are means, standard deviation, and range (in parenthesis).

| | 2009 | 2010 | 2011 |
|---|---------------------------|---------------------------|---------------------------|
| Hours of Effort (spring/fall) | 240/513 | 1232/949 | 1272/462 |
| Spring Dates of Sampling | April-8/June-5 | April-11/June-17 | April-1/June-2 |
| Spring Sturgeon Captured | 0 | 0 | 0 |
| Fall Dates of Sampling | Aug-5/Oct-9 | Aug-12/Oct-8 | Aug-18/Oct-7 |
| Fall sturgeon captured | 13 | 33 | 61 |
| Dates of Capture (first to last) | Aug-5/Oct-9 | Aug-17/Oct-8 | Aug-18/Oct-7 |
| Capture location (rkm) | 118-124 | 109-124 | 108-132 |
| Water Temperature (range for dates of capture, °C) | 19-30 | 19-26 | 20-25 |
| Verified Males (#) | 13 | 33 | 61 |
| Fork Length (cm) | 155±12(139-179) | 154±8 (138-181) | 158±10 (127-180) |
| Weight (kg) | 31±4 (25-39) | 30±6 (23-35) | 32±7 (19-51) |
| Girth (cm) | 71±4 (66-77) | 70±7 (59-82) | 73±5 (56-82) |
| Condition Factor | 0.83±0.13 (0.53- 1.00) | 0.82±0.08 (0.68- 0.99) | 0.85±0.11 (0.69- 1.03) |

Table 2. Results of 2011 Atlantic sturgeon semen analysis. Fish ID, collection and analysis date, amount of semen per sample, percent motility and density of sperm, and osmolality for each sample analyzed.

| Fish ID | Collection Date | Date Received | Amount (mL) | % Motility | Density ($\times 10^9$ /mL) | mOsmol/kg |
|---------|-----------------|---------------|-------------|------------|------------------------------|-----------|
| 0118 | 9-13-11 | 9-15-11 | 25 | 60 | 2.4 | 122 |
| 3436 | 9-14-11 | 9-16-11 | 15 | 0 | 4.1 | 123 |
| 3141 | 9-14-11 | 9-16-11 | 13 | 50 | 2.5 | 122 |
| 0644 | 9-14-11 | 9-16-11 | 15 | 80 | 2.4 | 108 |
| 0964 | 9-14-11 | 9-16-11 | 13 | 30 | 1.2 | 131 |
| 2204 | 9-14-11 | 9-16-11 | 10 | 0 | 2.8 | 120 |
| 5313 | 9-21-11 | 9-22-11 | 25 | 80 | 3.9 | 135 |
| 1419 | 9-21-11 | 9-22-11 | 27 | 80 | 3.0 | 108 |
| 1932 | 9-21-11 | 9-22-11 | 9 | 90 | 2.8 | 110 |
| 3167 | 9-21-11 | 9-22-11 | 10 | 80 | 5.6 | 127 |
| 2918 | 9-21-11 | 9-22-11 | 15 | 50 | 3.9 | 150 |
| 4169 | 10-3-11 | 10-4-11 | 25 | 80 | 5.0 | 136 |
| 2918 | 10-4-11 | 10-5-11 | 25 | 0 | 4.8 | 203 |
| 2564 | 10-4-11 | 10-5-11 | 25 | 80 | 3.3 | 116 |
| 2525 | 10-4-11 | 10-5-11 | 15 | 50 | 7.1 | 234 |

Table 3. Number of adult male Atlantic sturgeon tagged with ultrasonic tracking tags during the fall 2009-2011. Data show date and rkm where the fish were tagged, river departure and river return dates, and rkm traveled to in subsequent years.

| | 2009 | 2010 | 2011 |
|-------------------------|----------------|---------------|---------------|
| Number of Tagged Fish | 5 | 25 | 10 |
| Dates Tagged | Aug-21/Sept-18 | Aug-17/Oct-7 | Aug-18/Oct-4 |
| Tag Location (rkm) | 120-124 | 109-124 | 108-124 |
| Departure date | Oct-10/Nov-3 | Oct-19/Nov-3 | Oct-6/Nov-8 |
| Spring tag returns | | 0 | 8 |
| Spring residence | | | May-4/June-21 |
| Rkm reached (Spring) | | | 11-60 |
| Fall tag returns | | 1 | 15 |
| Fall return/depart date | | Aug-15/Oct-26 | Aug-10/Nov-3 |
| Rkm reached (Fall) | | 145+ | 133-142+ |

Chapter IV

Comparison of MS222 and Electronarcosis on Cortisol Levels in Juvenile Atlantic Sturgeon.

Abstract

Although stressful, invasive procedures on sturgeon (family *Acipenseridae*) are sometimes done without anesthesia. We examined cortisol levels in Atlantic sturgeon exposed to MS222, electronarcosis or no anesthetic 1 and 24 hr after a small incision mimicking tag implantation. We also determined the feasibility of using electronarcosis in the field and the effect of salinity on electronarcosis. Electronarcosis and MS222 anesthesia caused similar levels of plasma cortisol, but both levels were lower than in the no anesthetic group. Cortisol levels were similar between 1 and 24 hr after anesthesia but decreased in the no anesthesia group. Eighty-one adult male Atlantic sturgeon were implanted with tracking tags after electronarcosis demonstrating successful use in a freshwater field setting. Salinities above 1‰ interfere with anesthesia. We recommend electronarcosis because it avoids toxic chemicals, and anesthesia induction and recovery are instantaneous.

Key Words: Atlantic sturgeon, cortisol, electronarcosis, MS222, fish stress

Introduction

Investigators are increasingly conducting invasive procedures on sturgeon (family *Acipenseridae*) to track movements and monitor internal biology (internal tracking tag implantation, determining sex, gonad stage, and general health). Invasive procedures are stressful and increase cortisol levels (Barton 2002). In 2012 Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* was listed under the endangered species act. Increased understanding of Atlantic sturgeon life history is necessary for restoration which will require invasive procedures.

Traditionally, invasive procedures on sturgeon have been performed in the field, sometimes without anesthesia. There is debate over whether anesthetics create more stress than procedures such as tag implantation alone. Before listing in 2012 anesthetics were recommended but not required for invasive surgeries on Atlantic sturgeon (Damon-Randall 2010). Tricaine methanesulfonate (MS222), a chemical anesthetic, at various doses is commonly used for sturgeon (Damon-Randall 2010). MS222 is toxic, and requirements mandate fish be kept captive for a minimum of 21 days post treatment (Summerfelt and Smith 1990). Matsche (2011) measured cortisol levels after single exposures to varying MS222 concentrations. Electronarcosis, a physical anesthetic commonly used in salmonid research (Roth et al. 2003, Hudson et al. 2011) has been used on captive Atlantic sturgeon (Henyey et al. 2002). Managers are interested in the effectiveness of electronarcosis compared to commonly used MS222 on sturgeon species and whether anesthetics reduces stress (cortisol levels) compared to no anesthetic. This study examined cortisol levels in Atlantic sturgeon exposed to MS222, electronarcosis or no anesthetic during a small incision mimicking tag implantation. We also determined the feasibility of using electronarcosis in the field and the effect of salinity on electronarcosis.

Methods

Age 2 y (n = 20, 290-385 mm fork length, 85-235 g) and 3 y Atlantic sturgeon (n = 8, 380-466 mm fork length, 379-502 g), obtained from Maryland Department of Natural Resources, were maintained at the aquatics facility at Virginia Commonwealth University for a minimum of 6 months. All fish were handled routinely on a monthly basis to collect length and weight measurements. Four fish were randomly assigned to one of seven replicate 568 L flow through tanks two months prior to experimentation. Age classes were segregated. Tanks were maintained at 16-18°C under a 12:12 light/dark cycle, and fish were fed a pellet diet (Zeigler Bros Inc. product # 306540-18-44) once daily 5-7 times per week. Fish were fasted 24 h prior to treatment (Matsche 2011).

Individuals in each tank were randomly assigned as a control (no surgery), surgery with no anesthesia, surgery with MS222, or surgery with electronarcosis. To replicate an invasive procedure, a ~1 cm incision was made in the abdomen and closed with two double surgeon knots (Mohler 2004). The control, no anesthesia, and MS222 surgeries were conducted in 12 L tanks. Electronarcosis was conducted in a 16 L tank of similar length and width that was deeper to allow placement of cathode and anode screens.

To minimize possible daily-cyclic cortisol fluctuation two groups of four fish were processed per day: the first group started at 10:00 and the second at 10:30. Treatments were conducted on four consecutive Tuesdays (Virginia Commonwealth University IACUC AD20127).

All four fish per tank were removed simultaneously and placed in a treatment tank. The control fish were placed in the experimental tank for 1 minute and returned to their home tank.

The no anesthesia group was immobilized with straps on a stretcher during the procedure. The MS222 fish were exposed to 100 mg/L with 200mg/L of sodium bicarbonate, and time to reach level 4 anesthesia was recorded (Summerfelt and Smith 1990), after which fish were placed on a support stretcher for the surgical procedure. Stage 4 anesthesia is characterized by complete loss of spinal reflexes and slowed opercular movement (Summerfelt and Smith 1990). After surgery freshwater was run over the gills, and recovery time was recorded for fish to exhibit typical forward taxis before returning individuals to their home tank. Electronarcosis fish were placed on a support stretcher, and voltage (V) was increased (average 0.54 V/cm, range 0.46-0.57; average 0.08 amps, range 0.08-1.0) until level 4 anesthesia was reached although opercular motion never changed (Summerfelt and Smith 1990, Henyey et al. 2002). The fish's head was positioned toward the cathode (Hudson et al. 2011). Electronarcosis anesthesia was instantaneous so there were no induction or recovery times.

Blood was collected 1 h after removal from the holding tank when cortisol levels typically peak (Iwama et al. 2006, Matsche 2011), and a second sample was taken at 24 h. Samples (2.5 ml) were collected via caudal vein using a 21-gauge needle, and blood was transferred to heparinized vacutainer tubes with a plasma separation layer (BD Diagnostics, Franklin Lakes, New Jersey). Within 10 minutes of blood collection, vacutainers were gently inverted five times to mix the blood samples, placed on ice and spun in a refrigerated centrifuge at 1,000 X g for 15min. Plasma samples were stored at -80°C until cortisol extraction.

Cortisol was extracted using a Cortisol Express EIA Kit (Cayman Chemical Company, Ann Arbor, Michigan, Item No. 500370) following manufacturers protocols. Assays were run in triplicate at 1:2 and 1:4 dilutions or 3:1 concentration and read on a plate reader at a 410 nm

wavelength. Triplicate values were averaged. Samples were rerun if any of the triplicate readings varied by more than 10% of the samples average.

Since there were two size classes in the experiment, cortisol levels were compared against fish weight using linear regression. A block (by tank) one-way ANOVA and post-hoc Tukey test were run on the 1 h blood cortisol levels to determine differences between treatment types. One fish died soon after the 1 h blood draw. Therefore the block analysis was changed to a non-blocked one way ANOVA for the 24 h samples. A paired sample t-test was used to determine differences between 1 h and 24 h cortisol levels.

In order to determine if electronarcosis could be conducted feasibly on adult fish in the field an experimental setup was fabricated at Presquile National Wildlife Refuge, controlled by the US Fish and Wildlife Service. The setup consisted of a 244x61x36 cm tank with 0.6 cm mesh galvanized hardware cloth supported by adjustable plastic frames on each side of the tank. A 0-60VDC, 1.5A (BK Precision: Model 1623A) power supply was connected to the hardware cloth with alligator clips. The tank was filled with freshwater (tidal freshwater James River), and fish were placed with their head toward the cathode. The power supply was set to 0 V, and V was increased until stage 4 anesthesia was reached. Amperage, voltage, and distance between the cathode and anode were recorded. Captured fish were implanted with a Vemco V16-4x tracking tags (Mohler 2004). Fish fork length (FL), weight and water temperature were taken.

Results

MS222 anesthesia induction time averaged 6 min (range 4 -8 minutes) and recovery time 5 min (range 3-8 minutes). There was no relationship between cortisol levels and fish weight. One hour plasma cortisol levels indicated differences between treatments ($F_{3,18} = 13.04$, $p <$

0.0001, Fig. 1A). The no anesthetic group levels were four fold higher than controls ($p < 0.001$), and MS222 levels were over twice as high ($p < 0.05$). There was no difference between electronarcosis and control values or between MS222 and electronarcosis. At 24 h the no anesthesia group was significantly different from all other groups ($F_{3,23} = 3.506$, $p = 0.0315$, Figure 1B), but there was no difference between the two anesthetic treatments and controls. Levels did not decrease by 24 h in controls and the two anesthetic treatments but dropped from 10.3 to 6.8 ng/ml in the no anesthetic treatment (paired $t_6 = 2.061$, $p = 0.0425$). All fish ate within 2 h of the 24 h blood sample.

Varying salinity in captivity indicated that freshwater is required for electronarcosis: A mild sluggish behavior was observed in a salinity of 1 ppt, and there was no response in 2 ppt. When water temperatures were lowered to 12°C anesthesia and recovery using electronarcosis was still instantaneous.

Between 2011 and 2012, 81 adult male Atlantic sturgeon (average FL = 154 cm, range 127-207 cm, average weight = 41 kg weight range 21-74 kg) were implanted with tracking tags in late summer (18-27°C) using electronarcosis. In these larger fish induction and recovery were again instantaneous. Induction parameters averaged 0.16V/cm (range 0.14-0.19) and currents; averaged 0.08 amps (range 0.08-0.09). Procedures averaged 6 min (range 4-10 minutes). Of the nine fish tagged in 2011 eight returned in 2012.

Discussion

This study demonstrates that electronarcosis and MS222 anesthesia have equivalent effects on plasma cortisol levels after short duration surgical procedures. Levels were significantly elevated over controls for MS222 but not for electronarcosis, and surgery without

anesthetic had elevated levels four fold over controls. Control cortisol levels from this study are similar to values found by Matsche (2011) in captive juvenile Atlantic sturgeon and Barton et al. (2000) in captive juvenile pallid sturgeon *Scaphirhynchus albus*. However, our treatment results were lower than found by Matsche (2011) and Barton et al. (2000) for handling and confinement treatments, perhaps because our fish had been handled extensively prior to the experiment.

Although the two methods have equivalent effects on stress, we suggest there are multiple advantages to electronarcosis. First it allows instantaneous induction and recovery from anesthesia in various size fish and water temperatures from 12-27°C in a laboratory or field setting. MS222 anesthesia induction time increases in colder water, and increases with fish size (Henyey et al. 2002, Matsche 2011). For example, induction of stage 4 anesthesia in a 74 kg Atlantic sturgeon required 17 min at 14°C, which is a considerable delay in a field study requiring boat time. Although excessive electrical current causes erratic opercule movements and mouth protrusion in sturgeon, voltage can be decreased to safe levels immediately. MS222 overdose is harder to determine and more problematic to correct. Furthermore, MS222 introduces toxic chemicals into the fish and environment, particularly for large fish, and in some areas MS222 use is prohibited on sturgeon due to the chance of human consumption. Unfortunately, electronarcosis is ineffective in brackish water. However, Atlantic sturgeon commonly move between fresh and marine water and transfer of estuarine or marine sturgeon to a freshwater tank for brief procedures should not be harmful.

The National Institutes of Health allows surgical procedures without an anesthetic if a state of anesthesia "would defeat the purpose of the experiment" (NIH 1985). The National Oceanic and Atmospheric Administration now requires an anesthetic for invasive procedures on Atlantic sturgeon but not for other sturgeon species. Given the four fold increase in cortisol

levels with no anesthesia, it will be increasingly difficult to justify field procedures on non-anesthetized fish. Unfortunately, forgoing research will harm efforts to manage the species. Construction of a rig for electronarcosis is relatively inexpensive, and we recommend this technique for future field work on Atlantic and other sturgeon species.

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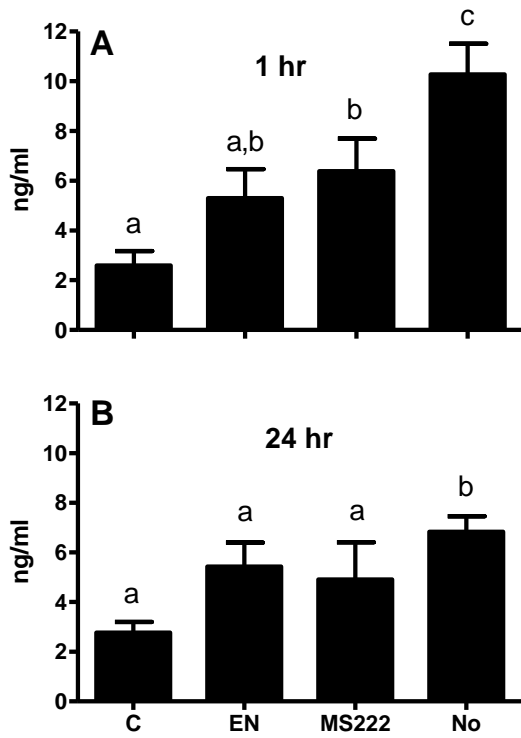


Figure 1. Plasma Cortisol levels (ng/ml) in juvenile Atlantic sturgeon 1 h (A) and 24 h (B) after surgery for controls, C, electroanesthesia, EN, MS222 and no anesthetic treatment (No). N = 6 for the 24 h MS222 treatment and 7 for all other treatments. Different letters above the bars indicate means that are significantly different (see text for p values).

Chapter V

Using energy dispersive x-ray fluorescence microchemistry to infer migratory life history of Atlantic sturgeon

Abstract

Atlantic sturgeon migrate between ocean and freshwater habitats to spawn, and juveniles spend several years in fresh/brackish water before returning to the ocean. Because strontium/calcium (Sr/Ca) ratios are diagnostic for freshwater and marine environments, we examined the utility of energy-dispersive x-ray fluorescence (EDXRF) to quantify Sr/Ca ratios of Atlantic sturgeon pectoral fin spines. Atlantic sturgeon spines from wild adults and experimental juveniles were analyzed along a linear transect from the primordium to the outermost point. To verify the technique hatchery juvenile Atlantic sturgeon were held in experimental tanks at <0.5, 13-15, or 33-35‰ and sampled after 5 months. Sr/Ca ratios of experimental hatchery fish increased with salinity, and Sr/Ca ratios in wild adults varied predictably along the measurement transect. However, the ratio decreased in the outermost region of the spine in mature fish collected during a return to freshwater for spawning. Therefore EDXRF is a useful tool to track individual movements of Atlantic sturgeons and other diadromous fish.

Key Words: Atlantic sturgeon, sturgeon spine, Sr/Ca ratio, diadromy, EDXRF

Introduction

Strontium/calcium (Sr/Ca) ratios of selected biogenic tissues (e.g. otoliths) are used to infer migration patterns between marine and freshwater environments for many fish species using laser ablation or wavelength dispersive microprobe analysis (Limburg 1995; Secor et al. 1995; Arai and Tsukamoto 1998; Allen et al. 2009). Elemental composition of fish tissues may be used to discriminate between marine and freshwater populations (or life history stages), determine links between natal rivers or nursery areas and adult stocks, and assess population structure in marine fishes (Sauer and Watabe 1989; Secor and Rooker 2000; Kraus and Secor 2004; Limburg et al. 2007). Energy dispersive x-ray fluorescence (EDXRF) is proposed as an alternative method that allows non-destructive and accurate determination of elemental composition of biogenic materials (Paiva et al. 1997, Lundblad et al. 2008). An EDXRF system works by detecting the x-ray energy released due to electrons changing shell layers of an atom after it has been excited by an x-ray laser. A silicone detector is used to determine the amount of energy released which is known for most elements and therefore percent mass of elements in an area can be quantified.

Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) is an anadromous, long-lived (60+ y), iteroparous, historically fishery-targeted species, making it a good candidate for Sr/Ca analysis. Atlantic sturgeon populations are depleted in the United States (Atlantic sturgeon status review team 2007). A better understanding of migration patterns and life history may aid in management and recovery efforts.

We evaluated the use of EDXRF analysis of pectoral fin spines from Atlantic sturgeon to potentially provide migratory and life history information. The ratio of Sr to Ca is positively correlated with environmental salinity (Limburg 1995); we quantified this ratio as distance

increases from the primordium in juveniles held under experimental salinity regimes and in wild adults during freshwater residence (Arai and Tsukamoto 1999; Secor and Rooker 2000).

Methods

We used 2 y old hatchery juvenile Atlantic sturgeon (295-340 mm fork length) to verify the instrument could detect variations of Sr/Ca ratios in the fish spines. The hatchery Atlantic sturgeon were acquired from the Maryland Department of Natural Resources Fisheries Division and held at the aquatics facility of Virginia Commonwealth University (VCU). The hatchery fish were maintained in freshwater prior to being moved to VCU and held at VCU for three months prior to salinity treatment. Richmond city water was filtered to remove chloramines, and salinity regimes were prepared with Instant Ocean[®] sea salt. Salinity was monitored using a refractometer (Bath et al. 1999). The ion ratio of Instant Ocean[®] sea salt mimics typical saltwater by 98.5% (US Aquatics Consumer Support). The three treatment tanks were maintained at identical temperature (16-18°C) and fed an identical diet (Zeigler Bros Inc. product # 306540-18-44) because both affect Sr/Ca uptake (Fowler 1995; Secor et al. 1995; Gallahar and Kingsford 1996; Bath et al. 1999).

A section from the left leading fin spine was taken from each fish for pre-treatment analysis. The following day we measured fish for fork length and placed them in experimental tanks. The fish were separated into three treatment groups with three fish per treatment, freshwater (<0.5‰), brackish (13-15‰) or saltwater (33-35‰) tanks for 5 months. Fish were measured, and the right leading pectoral spine was removed (VCU IACUC AT20127) at the termination of the experiment.

Pectoral fin spines were removed from carcasses of 13 recently killed wild adult male Atlantic sturgeon found in September, 2008 and 2009 during a putative spawning period in the

freshwater portion of the James River, Virginia. The carcasses were found by researchers examining the shoreline for Atlantic sturgeon mortalities. These fish were confirmed as adult males due to fork length measurement and having fully developed gonads.

A 2 mm thick section of the leading fin spine was cut within 1 cm of the articulation point with an isomet saw. A section from the left spine was used when available; however, the right spine was used if the left spine was not present. Extreme care was taken to insure the sample section was cut orthogonally. Soft tissue on the spine was removed with a fine brush. The spine was then rinsed with deionized water and air-dried.

Samples were analyzed for elemental analysis on a Horiba X-Ray Guide Tube XGT-7000V EDXRF microscope with 50 kV of energy at 1 mA with a 100 μm probe held under vacuum. Each sample point was $\sim 100 \mu\text{m}$ in diameter. The machine was calibrated using protocols and samples provided by the microscope's manufacturer. To support the sample with minimal background, plastic wrap (Fisher Scientific) was placed on a flat stage with a 5 cm x 5 cm hole in the middle of the stage. Two millimeter thick spine samples were attached to the plastic wrap using double sided tape. Analysis with a copper plate backing indicated 1.9 mm thick samples were sufficient to block the copper signature, i.e. laser excitations are restricted to the spine sample. Sampling points were measured equidistant along a linear transect across the spine section for 30 s per point, and each point measurement was repeated to verify precision (Figure 1). For all samples extreme care was taken to ensure the most peripheral portion of the spine section was sampled. The Sr/Ca ratio from both transects was averaged for each point.

Results

The salt and brackish water tanks had one fatality each leaving an n=2 for these treatments. After 5 months in the aquatic center, the average fork length of the hatchery fish increased 31 mm (23-50 mm). An ANOVA ($F=1.62$, $p=0.13$) indicated no significant difference in the Sr/Ca ratio among the pre-treatment samples (Figure 2A). The ratios of freshwater-control fish stayed flat between 0.2×10^{-3} and 0.3×10^{-3} (Figure 2B). In the salt and brackish treatments Sr/Ca values increased toward the edges of the spine and leveled off. The brackish water treatment maximum ratio was 0.9×10^{-3} (greater than 3x freshwater values), and the saltwater treatment maximum was 1.7×10^{-3} (a further doubling compared to brackish water). The lack of overlap in Sr/Ca ratios between treatments is a strong result and additional statistics are not necessary (Yoccu 1991). Variation in the replicate point runs averaged 3.7% and ranged between 0-6% indicating reasonable precision.

The spines of all 13 wild fish had similar patterns, and transects from three representative individuals are shown in Figure 2C. Mean Sr/Ca ratios in wild Atlantic sturgeon increased from 0.3×10^{-3} at the spine primordium to 1.5×10^{-3} at the periphery (paired $t_{12}=-16.0949$, $p<0.0005$). Ratios decreased at the outermost portion of the spine consistent with a return to a freshwater environment (Figure 2C).

Discussion

This study successfully demonstrates the ability of EDXRF to indicate an ontogenetic change in Sr/Ca ratios of Atlantic sturgeon consistent with migration across an environmental salinity gradient. It has an advantage over other methods because it is non-destructive to the sample. However, similar results have been found in green sturgeon (*A. medirostris*) using laser ablation on spines and Russian sturgeon (*A. guldenstadi*) using wavelength dispersive x-ray electron microprobe analysis on otoliths (Arai and Miyazaki 2001, Allen et al. 2009). Ratios in

experimental juveniles in this study increased with salinity indicating that 5 months and 23 mm of growth are sufficient for a salinity signature to imprint on an Atlantic sturgeon spine. By comparing annuli (Balazik et al. 2010) of the 13 wild fish with the position of increased Sr/Ca ratios our data suggest Atlantic sturgeon out migrate from natal rivers between 1 to 4 y of age. The ratio increased by age 4 for the 14 y old, by age 3 for the 16 y old, and by age 2 for the 19 y old (Figure 2C). These findings agree with previous catch data on Atlantic sturgeon outmigration (Bain 1997, Kynard and Horgan 2001). Sr/Ca ratio decreased at the spine periphery in the three adult male samples collected in freshwater, indicating that water chemistry can be imprinted on the spine during inward migration. With increased sampling point density this new technique could be used to determine natal immigration to brackish environments, further movement from brackish to ocean environments and perhaps even spawning events.

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List of Figures

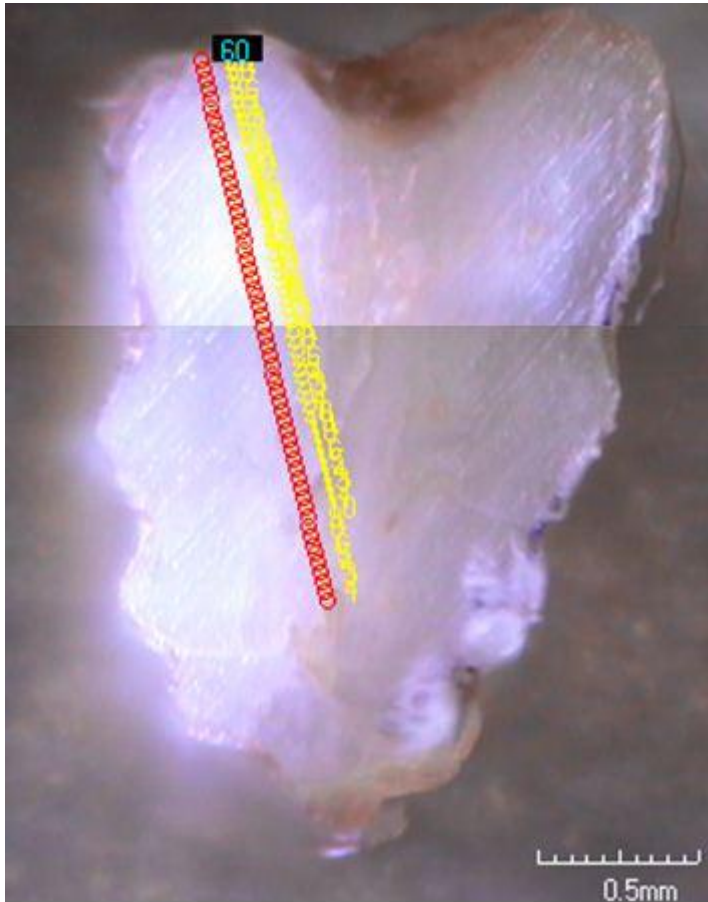


Figure 1. Photograph of Sr/Ca analysis points on an Atlantic sturgeon pectoral spine. The red circles show loci where Sr/Ca was analyzed, and the yellow number (0-60 in this case) is the point number along the transect. Point one is at the primordium and point 60 is at the spine edge. Each point was measured twice and the average was used for analysis.

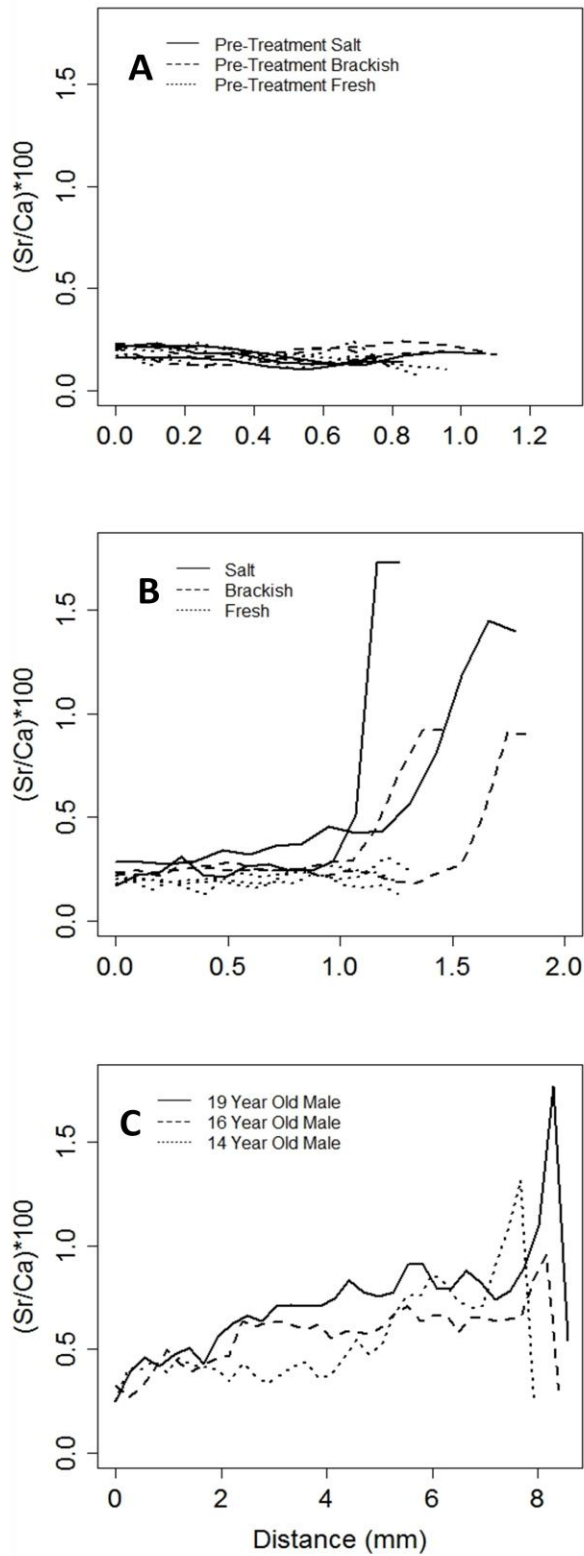


Figure 2. The percent mass of Sr/Ca ratios at different distances from the primordium of Atlantic sturgeon spines. A. The percent mass of Sr/Ca ratios of experimental juveniles prior to salinity level treatment. B. The percent mass of Sr/Ca ratios of experimental juveniles maintained at different salinity levels. C. The percent mass of Sr/Ca ratios of three representative wild adult male Atlantic sturgeon captured in the James River.

Chapter VI

Technical Report: Current Status of James River Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*)

Abstract

Atlantic sturgeon have been severely depleted across their entire range due to habitat destruction, pollution, and overfishing. Currently fisheries managers and researchers are developing plans to help Atlantic sturgeon recover. In order to be most effective management needs information about the current state of the managed species. The purpose of this document is to provide management with the best available data describing the status of Atlantic sturgeon in the James River. This report will cover current knowledge of reproduction, distribution of various life stages, population numbers, threats, field techniques used to monitor the population, and recommended research needs. With these new data managers will be able to construct better management plans addressing issues in the James River, which should help recovery of Atlantic sturgeon populations.

Introduction

Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* were once plentiful throughout the Chesapeake Bay (Hildebrand and Schroeder 1928, Murawski and Pacheco 1977). Pollution, habitat destruction, and overfishing lead to the Atlantic sturgeon decline throughout the entire Chesapeake Bay (Smith 1985, ASSRT 2007). Chesapeake Bay Atlantic sturgeon were considered functionally extirpated at the end of the 20th century (Secor 1996, Speir and O'Connell 1996, Grogan and Boreman, 1998). In 1997 a reward program targeting hatchery released Atlantic sturgeon was conducted in the Chesapeake Bay. Hundreds of hatchery Atlantic sturgeon were recaptured but many wild origin fish were collected (Secor et al. 2000). Microsatellite analysis of fin clips from wild origin fish determined there was a distinct population segment (DPS) reproducing in the Chesapeake Bay (King et al. 2001).

Very little is known about the distinct population of Atlantic sturgeon reproducing in the Chesapeake Bay. There has been verified reproduction in the James and York Rivers; however, evidence suggests reproduction is also occurring in the Rappahannock and Nanticoke Rivers.

Objective

Atlantic sturgeon research has been conducted in various parts of the Chesapeake Bay. This document is intended to be an overview of work conducted in the James River and provide information for management. This report will cover: distribution of Atlantic sturgeon throughout the James River, information on spawning season, area, and frequency, population estimation, threats to restoration, field techniques used to collect fish, and research needs.

Study Site

The James River drains about 26,000 km² as it flows east about 540 rkm to the Chesapeake Bay. The river is tidally influenced up to the city of Richmond located at rkm 155.

Current Atlantic sturgeon research is focused on the tidal portion but evidence shows that fish infiltrate the non-tidal portion of the river (Matthew Balazik, personal observation).

James River Atlantic sturgeon research is mainly conducted in two areas: Presquile National Wildlife Refuge (Presquile) and Burwell Bay (Figure 1). A Fisheries Resource Grant (FRG) funded by Virginia Sea Grant has been ongoing in Burwell Bay from 2005-2012. The objectives of the Burwell Bay research have varied from year to year but provide Atlantic sturgeon collection records (mostly juveniles) throughout the study period. During the FRG study various size mesh nets were used so all size class fish would be sampled. Research conducted at Presquile focused on the collection of adult Atlantic sturgeon in the freshwater portion of the river. Presquile work started in 2007 but records were poorly maintained prior to 2009; therefore, data from 2009 and later are discussed.

Life History/Distribution

Atlantic sturgeon have a complex life history that can be broken up to various stages (Bain 1997). For this document Atlantic sturgeon life history is divided into four stages: mature fish, young-of-year (YOY), migratory juveniles, and subadults.

Sexually mature fish

Sexually mature fish are characterized as either having a fork length (FL) > 129 (Balazik et al. 2012b) or visual inspection of developed gonads (Mohler 2004).

James River Reproduction

Historically large numbers of James River Atlantic sturgeon reproduced in the spring of the year from April to June (Hildebrand and Schroeder 1928, Smith 1985, Woodlief 1985).

After the fishery collapsed in the early 20th century there has been little sign of recovery for spring spawning Atlantic sturgeon. Since 2009 two adult Atlantic sturgeon collections have been

collected in the freshwater portion of the James River in the spring. One collection included a recently spawned female and the other was a male. Telemetry data showed the male fish stayed below rkm 85. Most freshwater sampling effort has been conducted upstream of rkm 105. The FRG has collected 81 sexually mature Atlantic sturgeon at Burwell Bay from April to June. No evidence suggests Atlantic sturgeon collected at Burwell Bay moved upstream to freshwater gill net sampling areas. It is possible Atlantic sturgeon are spawning in the spring, but reproduction would have to occur downstream of freshwater sampling areas.

In August 2007 Atlantic sturgeon were seen breaching in the main stem James River and major tributaries such as the Appomattox and Chickahominy Rivers. Sturgeon breaching had not been documented in the James River for decades. Observations of breaching prompted Atlantic sturgeon research in the James River in the fall season. From 2007 to 2011 there was substantial empirical evidence collected indicating Atlantic sturgeon spawned in the fall (Balazik et al. 2012b). Fall season spawning was confirmed in 2012 when a female Atlantic sturgeon was collected while releasing eggs. Within 5 h 15 males were caught in the surrounding area expelling milt. Telemetry data show adult male Atlantic sturgeon enter the Chesapeake Bay in late July/early August and leave the river by November. One-hundred ninety-nine Atlantic sturgeon have been collected during the fall spawning season. Sex determination of males is simple because most release milt while handled. Two confirmed females were collected, one on September 9 2011 and one on September 14 2012. Sex determination was unsuccessful on four fish. All four fish, captured between September 9 and September 17, had condition factors greater than all 180 confirmed males (Balazik et al. 2012a).

By early September most males stage between rkm 100 and 125, which usually coincides with the location of the salt wedge. During September to early October male fish migrate upstream above rkm 145 (extent of passive tracking array) and drop back down to staging areas. Adult Atlantic sturgeon have been seen in the fall line rapids. These upstream migrations can be from 1 to 12 d long. The fall line and area ~300 m below has ideal spawning habitat for Atlantic sturgeon (Ryder 1890, Smith 1985). Historically Native Americans harvested Atlantic sturgeon in the fall-line rapids during the spring (Woodlief 1985). Collections of females and tracking data suggest fall spawning occurs around the fall line in middle to late September. In order to verify Atlantic sturgeon spawning egg mats and larvae d-nets should be set around the fall line.

Sex Ratio

The sex ratio of fall season gill net collections is 90:1 (Balazik et al. 2012a) and 34:1 for boat mortalities (Balazik et al. 2012c). The skewed sex ratio trend (80:1) is also seen in spring season Burwell Bay collections. The skew in sex ratio might be due to several factors. Males may spend over two months in our sampling areas (Balazik 2012c), while females spend much less time in these sampling areas (Van Eenennaam et al. 1996, Bain 1997). Male James River Atlantic sturgeon become sexually mature around age 10 y and females mature around age 15 y (Balazik 2012b). Most (83%) fall season adult male sturgeon are less than age 15 y. It is possible the sudden resurgence of Atlantic sturgeon in the James River in 2007 is from a highly successful spawning event in the late 1990s or ASMFC moratorium (1998) and females have yet to sexually mature. Gear choice may affect female catch rates. Females grow larger than males (Dovel and Berggren 1983, Stevenson and Secor 1999) and historically commercial fishers used 41 cm stretch mesh net to target females (Ryder 1890). The largest mesh currently used is 33 cm. The confirmed and four hypothesized females were too large for the net webbing and

luckily wrapped themselves in the net. It is possible that using larger mesh gill nets collections of females would increase. There is also a possibility that something causes death prior to females becoming sexually mature.

Spawning Frequency

Atlantic sturgeon males are described as spawning every 1-5 y and females 3-5 y (Smith 1985). Tracking data showed that 89% of male Atlantic sturgeon internally tagged in 2011 returned in 2012. Only 64% of externally tagged males returned the subsequent year. Lower returns of externally tagged fish may be due tag loss. Nothing can be inferred from spawning frequency of females due to lack of collections and tracking data.

Young-of-year (YOY)

The YOY life stage is characterized as the period before the first annulus is formed. Atlantic sturgeon annulus formation occurs from February to April (Stevenson and Secor 1999). Because of the lack of data to distinguish spring and fall born fish we are combining the two groups.

The YOY life stage is poorly understood for Atlantic sturgeon throughout their range (ASSRT 2007). Collections from the Delaware River show YOY Atlantic sturgeon inhabit freshwater areas within a few rkm upstream of the salt wedge (personal communication, Matthew Fisher, Delaware Department of Natural Resources). For our purposes we consider YOY fish to be < 31 cm fork length (FL), which was the shortest age 1 y old Atlantic sturgeon aged by Balazik et al. (2012b). The only Atlantic sturgeon YOY collections in the James River were by trawls in the Virginia Institute of Marine Science's (VIMS) trawl survey (n = 11) and one by the Chesapeake Bay Foundation Education Outreach Group (Table 1, Figure 2). With the limited YOY data available not much can be determined about distribution and movements in

the James River. Collection data shows Atlantic sturgeon YOY inhabit the James River year round (Table 1) and congregate in the main channel (Figure 2). Further research is needed to determine YOY Atlantic sturgeon movements and habitat preferences.

Migratory Juveniles

Migratory juveniles are characterized as being sexually immature fish that frequently return to the James River estuary seasonally each year (Bain 1997), entering the river around March and leaving around November. It is not known where the fish overwinter. Migratory juveniles tend to be age 1-5 y old fish, 31-<85 cm FL (Balazik et al. 2012b).

Thirty migratory juvenile Atlantic sturgeon have been collected in the VIMS trawl survey from 1964 to 2005 (Figure 3). The VIMS trawl survey covers most of the tidal James River. The lack of collections in the upstream area suggests migratory juveniles mostly inhabit areas downstream of rkm 75 (Figure 3). Tracking data shows migratory juveniles (n = 8) inhabit the lower part of the river; however one fish did move upstream to rkm 90 (unpublished data). One migratory juvenile was collected in July of 2008 at rkm 105.

In 2005 a FRG was funded to search for Atlantic sturgeon utilizing the James River. Unsuccessful at first the investigators went to other commercial fishers for advice on Atlantic sturgeon locations. The FRG moved efforts to the Burwell Bay area (Figure 1) and was successful in catching migratory juvenile Atlantic sturgeon. The FRG caught 358 migratory juvenile Atlantic sturgeon with 14 recaptures. Preliminary data suggests that Burwell Bay is a nursery area for migratory juveniles. Increased tracking and gill net efforts throughout the river are required to better understand migratory juvenile numbers and distribution.

Subadults

Subadults are characterized and being sexually immature fish that spend most of their time in coastal areas and infrequently inhabit riverine areas (Bain 1997, Stevenson and Secor 1999, Balazik et al. 2012b, Balazik et al. 2012d). Based on length at age data James River fish from this group are age 6-9 y, FL 85-<129 (Balazik et al. 2012b). During the FRG program 158 subadults were collected. Sparse tracking data suggests fish in this size class do not stay in the James River long or return on a yearly basis. As with migratory juveniles increased telemetry and sampling effort is required to better understand subadult distribution in the James River.

Population

Genetics

Because of spawning site fidelity DPS have formed along the Atlantic sturgeon's range (Wirgin et al. 2000, King et al. 2001). Over 95% of genetic samples from 145 fall spawning Atlantic sturgeon were determined to be of James River origin. During the FRG over 500 genetics clips have been collected from Atlantic sturgeon ranging from migratory juveniles to mature fish. Processing of the FRG samples would provide a valuable mix stock analysis which would help management of all DPS.

Catch Index

Presquile collections were 15 in 2009, 32 in 2010, 77 in 2011, and 75 in 2012. A generalized liner model with bias correction showed there was no significant difference in catch-per-unit-effort (CPUE) across all four years (Figure 3); CPUE was determined to be fish caught per 100 m² of net fished per hour. The catch index (a product of population size) indicates there has not been any significant change in population size over the past 4 y.

“Rough” Population estimate

A Lincoln-Peterson mark-recapture model with Chapman Estimator was used to generate a population estimate for the sexually mature adult male James River Atlantic sturgeon. Using catch data from 2011 and 2012 the model estimates the James River population has 2760 adult males. The estimate does not take into account fish using the Chickahominy or Appomattox River. The population estimate should be used with caution because the data violate assumptions of the model. However, the population estimate is likely more accurate than the estimated 300 adults used by current management.

Threats

Bycatch of Atlantic sturgeon has been intensely studied by various management groups in the marine environment (Stein et al. 2004). In the James River various commercial fisheries may interact with Atlantic sturgeon during river residence. We will also discuss ship strikes and predation by introduced species on Atlantic sturgeon in the James River.

White Perch

The white perch *Morone americana* fishery typically operates from December to February between rkm 50 and 75. Atlantic sturgeon typically inhabiting the river at this time are YOY and some small age 1 y fish. White perch nets are typically 7.6 cm stretch mesh and set cross tide with excess floats attached to the float line to lift the net off the bottom. Since small Atlantic sturgeon tend to stay on the bottom (Ryder 1890), interactions with white perch nets should be uncommon. If an Atlantic sturgeon did get caught in a white perch net survival should be high due to cold water temperatures. In 2008-2009 a FRG grant was funded to monitor Atlantic sturgeon bycatch in the white perch fishery. During the entire season two Atlantic sturgeon were caught. One fish (91 cm FL) was sickly and just pressed against the mesh of the net by the flowing tide. The other fish (65 cm FL) was entangled/gilled by the net but was

healthy enough to be used in a telemetry study. There is no evidence to suggest the white perch fishery is a threat to Atlantic sturgeon in the James River.

Striped Bass

For several years the FRG focused on bycatch of Atlantic sturgeon in the striped bass *Morone saxatilis* fishery. The results showed that Atlantic sturgeon are susceptible to striped bass gill nets, but the results may be misleading. The timing and locations of the James River striped bass fishery makes bycatch of Atlantic sturgeon an uncommon occurrence. Since the FRG was started no commercial striped bass commercial fishers worked in the Burwell Bay area because of low catch numbers. The 2012 FRG study design used striped bass gear in the Burwell Bay area and areas outside of Burwell Bay where striped bass fisheries typically take place. The nets mimicking the striped bass fishery caught one Atlantic sturgeon compared to 30 in the Burwell Bay area. Time of year must also be considered. The commercial striped bass season opens in February and many commercial fishers in the James River fill their quota by late March. Atlantic sturgeon typically are not in the James River when striped bass are targeted.

Blue catfish

A blue catfish *Ictalurus furcatus* hoop net fishery operates year round, mostly in the freshwater portion of the James River. The nets consist of 5-8 hoops attached by 2.5 cm multitwine mesh making a cylinder with a hole in one end. The hoops have a diameter of 1.8 m. Blue catfish swim into the nets and get funneled to a open area cod-end. Rarely small Atlantic sturgeon (< 50 cm) swim into these fish traps. If Atlantic sturgeon do swim into these hoop nets the open area around the cod-end allows the fish to swim and not be suffocated. I have personally witnessed hundreds of hoop nets fished in the James River and never encountered an Atlantic sturgeon.

Ship-Strikes

Ship-strikes on adult Atlantic sturgeon are a common occurrence in the James River. Since 2007 thirty-five adult Atlantic sturgeon carcasses have been discovered in the James River. The number of ship-strikes discovered has decreased since 2010 likely due to decreased deep-draft traffic (Balazik et al. 2012c). Ship-strikes are a threat to adult Atlantic sturgeon spawning in the James River, but the degree is unknown. Research is required to determine how many Atlantic sturgeon are being killed and how Atlantic sturgeon react to boats (Balazik et al. 2012c). Ship-strikes on juvenile Atlantic sturgeon have been documented in the Delaware River (Brown and Murphy 2010). No ship-strike mortalities of sexually immature Atlantic sturgeon have been documented in the James River.

Introduced Species

The introduction of piscivorous catfish, blue catfish, channel catfish *I. punctatus*, and flathead catfish *Pylodictis olivaris*, to the James River pose a threat to larval and YOY Atlantic sturgeon. Flowers et al. (2011) describes predation of YOY Atlantic sturgeon by flathead catfish. Although not documented by researchers, commercial fishers describe blue catfish regurgitating YOY Atlantic sturgeon. Gut content work of introduced-piscivorous catfish is required to further understand this possible threat.

Field Techniques

Since 2007 efforts targeting adult Atlantic sturgeon have been conducted in the freshwater portion of the James River. Since 2009 catch records document numbers and fish condition. Here I will give an overview of catch results and describe our use of electronarcosis as an anesthetic in the field.

Gill Nets

Four different size nets (25 cm, 26 cm, 30 cm, and 33 cm stretch mesh) have been used to collect adult Atlantic sturgeon during the fall sampling season (Table 2). The 25, 26, and 30 cm stretch mesh webbing was single strand monofilament (0.9 mm width) while the 33 cm stretch mesh net was multimonomofilament (3 strands of 0.4 mm width braided together). All nets were "hung on a half" meaning a 25 cm stretch mesh net had a mesh sown to the lines every 12.5 cm. The 25 cm net was not fished much from 2009 to 2011 due to the risk of fish mortality (Table 2). Because of improved resources and knowledge the 25 cm net was used routinely in 2012. Nets typically soaked for 1 h between pulls but due to problems encountered sometimes soak times were greater. The CPUE was fish caught per 100 m² of net fished per hour.

There was no significant difference in the size (FL) of fish caught by each net (Figure 5). The 33 cm net caught the largest range of fish (Figure 5). The 25 cm net had the highest CPUE but also the highest percent mortality and fish that needed to be revived (Table 2).

Most fish collected were males around 155 cm FL (Figure 2). The morphology of these males allows them to push their heads through the 25-26 cm openings and get caught by the cleithrum. Once caught the net squeezes the gills and the fish suffocates. Multimonomofilament nets are "looser" compared to single monofilament nets. Multimonomofilament nets entangle fish more often than gilling. Entanglement has a lower chance of restricting gill movements. Extra caution should be used when using 25 cm nets compared to larger mesh sizes, but with proper knowledge and preparation they can be used successfully in the field.

Electronarcosis

Electronarcosis, a physical anesthetic commonly used in salmonid research (Roth et al. 2003, Hudson et al. 2011), has been used on captive Atlantic sturgeon in a laboratory setting (Henyey et al. 2002). In 2011 electronarcosis was used for general anesthesia for internal tag

implantation in a field setting (Balazik 2012). In 2011 and 2012 electronarcosis was used as an anesthetic to implant 81 adult male Atlantic sturgeon with transmitters. Anesthesia and recovery was instantaneous for all fish. Telemetry data showed that all fish survived and eight of nine fish tagged in 2011 returned in 2012. Surgery procedures average 6 min (range 4-10 min).

Electronarcosis is quicker and safer compared to tricaine methanesulfonate (MS22) or other chemical anesthetics. Electronarcosis does not introduce more toxic chemicals to the environment unlike MS222. Electronarcosis setups are portable and can be made for less than \$400. This form of anesthetic should be used more in field settings (Balazik 2012).

Research Recommendations

Various research needs are required to help management make informed decisions to help Atlantic sturgeon recovery in the James River. Spawning success is the building block that drives species recovery (Gross et al. 2002). Efforts must be put towards determining why so few females are collected during spawning runs, i.e. are low female catch numbers due to collection techniques or low incidence of female spawning. Identification of spawning habitat (spring and fall) is paramount for species recovery in the James River. Once found spawning grounds can be protected and possibly enhanced to increase spawning success, i.e. reduce invasive predators in the area. Discovery of spawning grounds will also provide genetic samples from known spawning season fish. Determining if spring and fall season fish are genetically distinct from each other may modify management decisions. It is possible spring spawning fish are in decline while fall spawning fish are recovering. Two disparate recovery plans may be necessary for the James River.

Determining YOY distribution in the river will be easier if they are tracked while radiating away from spawning grounds. Once YOY congregation areas are found they can be protected, and we can determine the impact of introduced predators on Atlantic sturgeon.

Having a functional, properly placed and maintained passive tracking array is the first step to finding spawning habitat. Properly located passive receivers will provide a general location of spawning habitats, which will enable fine scale searching (egg mats, snorkeling) and spawning habitat discovery. The James River currently has a passive tracking array but more receivers are required for spawning ground discovery.

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List of Tables

Table 1. Date, length, and collection location of young-of-year Atlantic sturgeon collected in the Virginia Institute of Marine Science's trawl survey.

| Collection | Date | FL (mm) | Latitude | Longitude | Comments |
|------------|------------|---------|----------|-----------|-----------|
| | 8/10/1972 | 113 | 37.21655 | -76.83308 | VIMS |
| | 8/10/1972 | 129 | 37.21665 | -76.83315 | VIMS |
| | 8/10/1972 | 100 | 37.21675 | -76.83304 | VIMS |
| | 1/29/1975 | 129 | 37.53500 | -76.95333 | VIMS |
| | 2/6/1975 | 200 | 37.35833 | -77.30335 | VIMS |
| | 6/30/1978 | 112 | 37.23499 | -76.94178 | VIMS |
| | 6/30/1978 | 115 | 37.23169 | -76.94177 | VIMS |
| | 12/11/1979 | 85 | 37.21000 | -76.69679 | VIMS |
| | 4/15/1997 | 161 | 37.55216 | -76.86152 | VIMS |
| | 4/25/1997 | 225 | 37.19566 | -76.77250 | VIMS |
| | 3/1/2004 | 155 | 37.30852 | -77.16333 | CBF trawl |
| | 3/15/2004 | 170 | 37.20434 | -76.78025 | VIMS |

Table 2. Net characteristics, effort, and collections of various size gill nets used to collect adult Atlantic sturgeon in the James River. Catch-per-unit-effort (CPUE) is fish caught per 100 m² of net fished per hour.

| Stretch Mesh Size (cm) | Meshes Deep | 100 m ² Hours Fished | Fish Caught | CPUE | Revived Fish | Dead Fish |
|---------------------------|----------------|---------------------------------------|----------------|------|-----------------|-----------|
| 25 | 20 | 39 | 15 | 0.38 | 3 | 2 |
| 26 | 16 | 267 | 86 | 0.32 | 5 | 1 |
| 30 | 16 | 174 | 51 | 0.29 | 0 | 0 |
| 33 | 16 | 156 | 47 | 0.30 | 0 | 0 |

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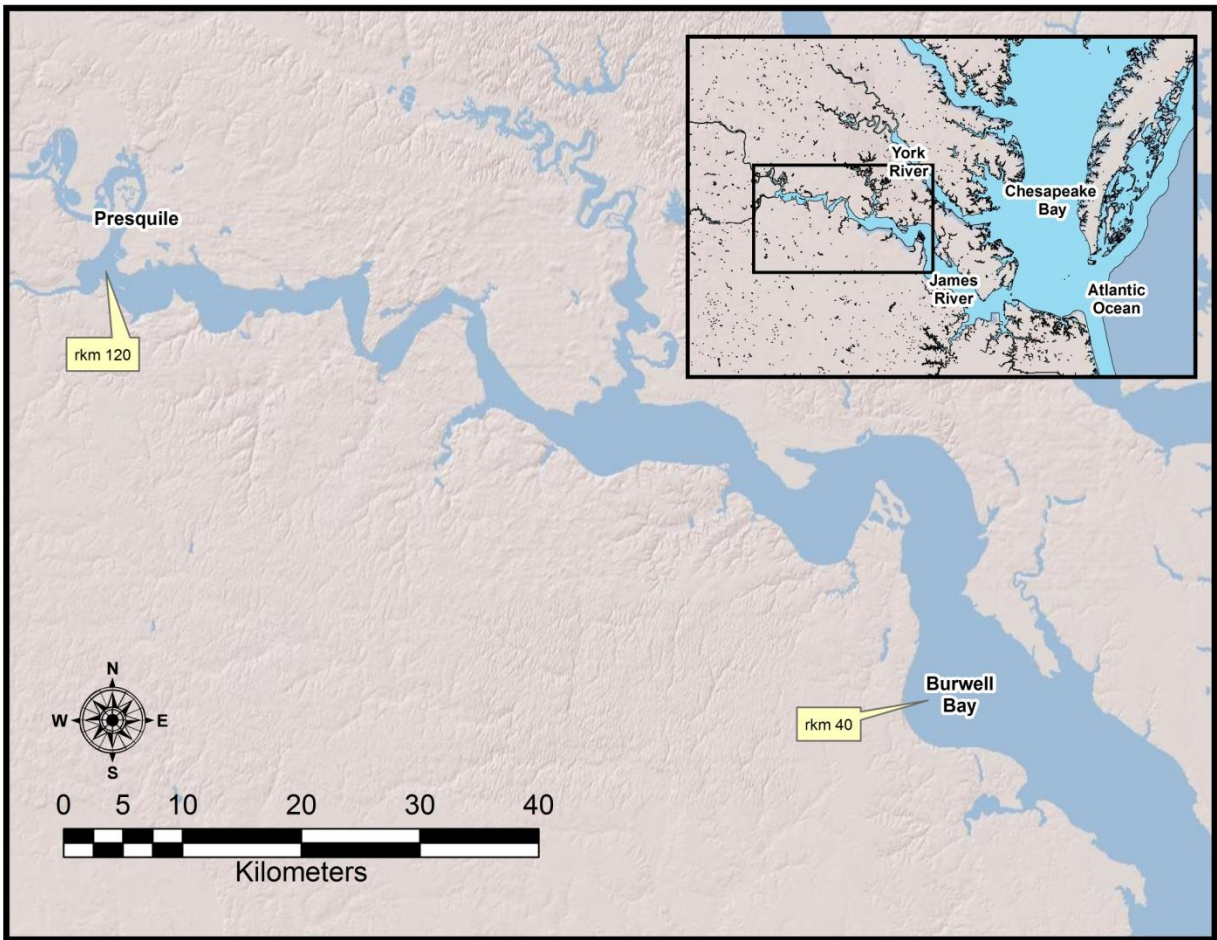


Figure 1. Map showing locations of James River Atlantic sturgeon sampling areas, Burwell Bay (rkm 40) and Presquile National Wildlife Refuge (rkm 120).

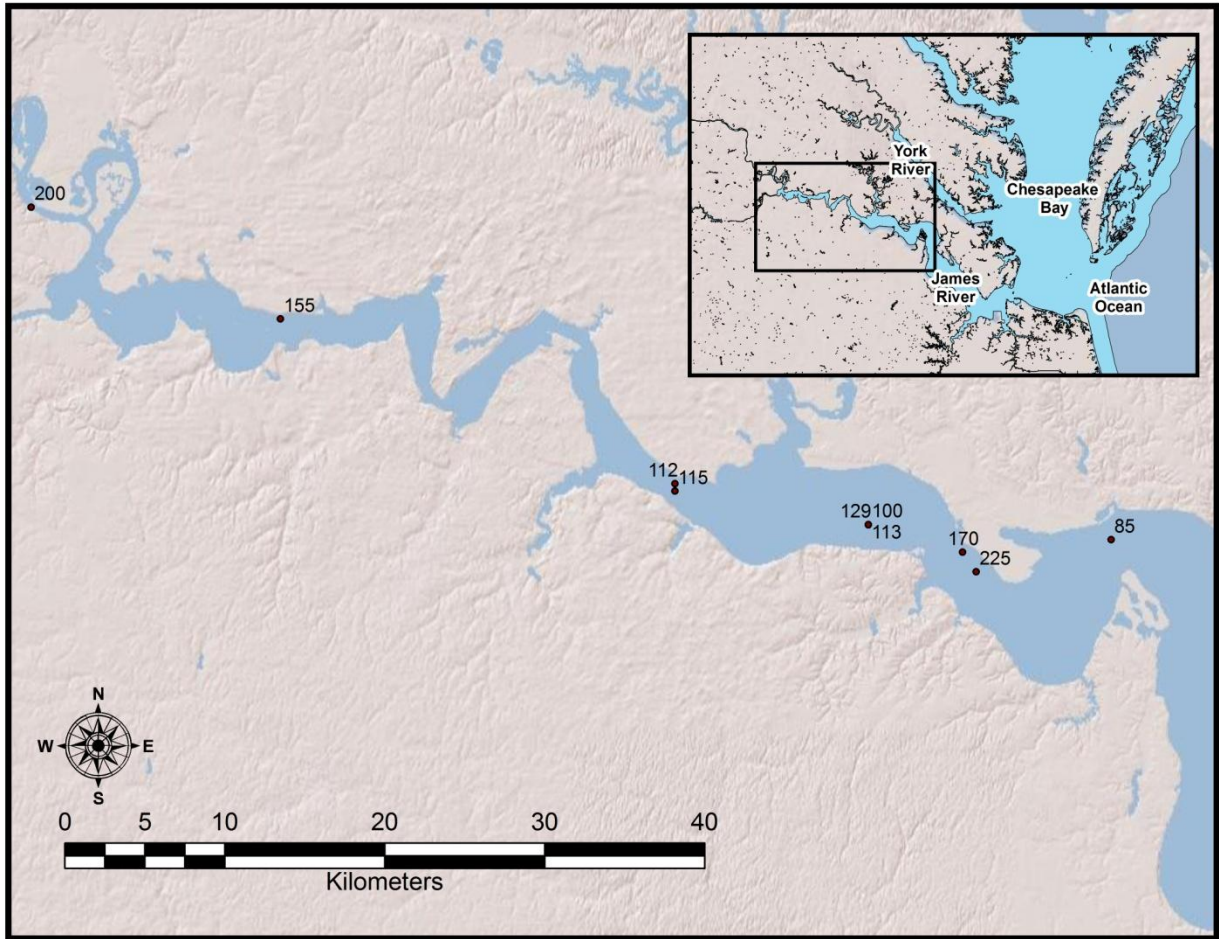


Figure 2. Map showing Virginia Institute of Marine Science trawl collections of young-of-the-year Atlantic sturgeon. Numbers are fork length in mm.

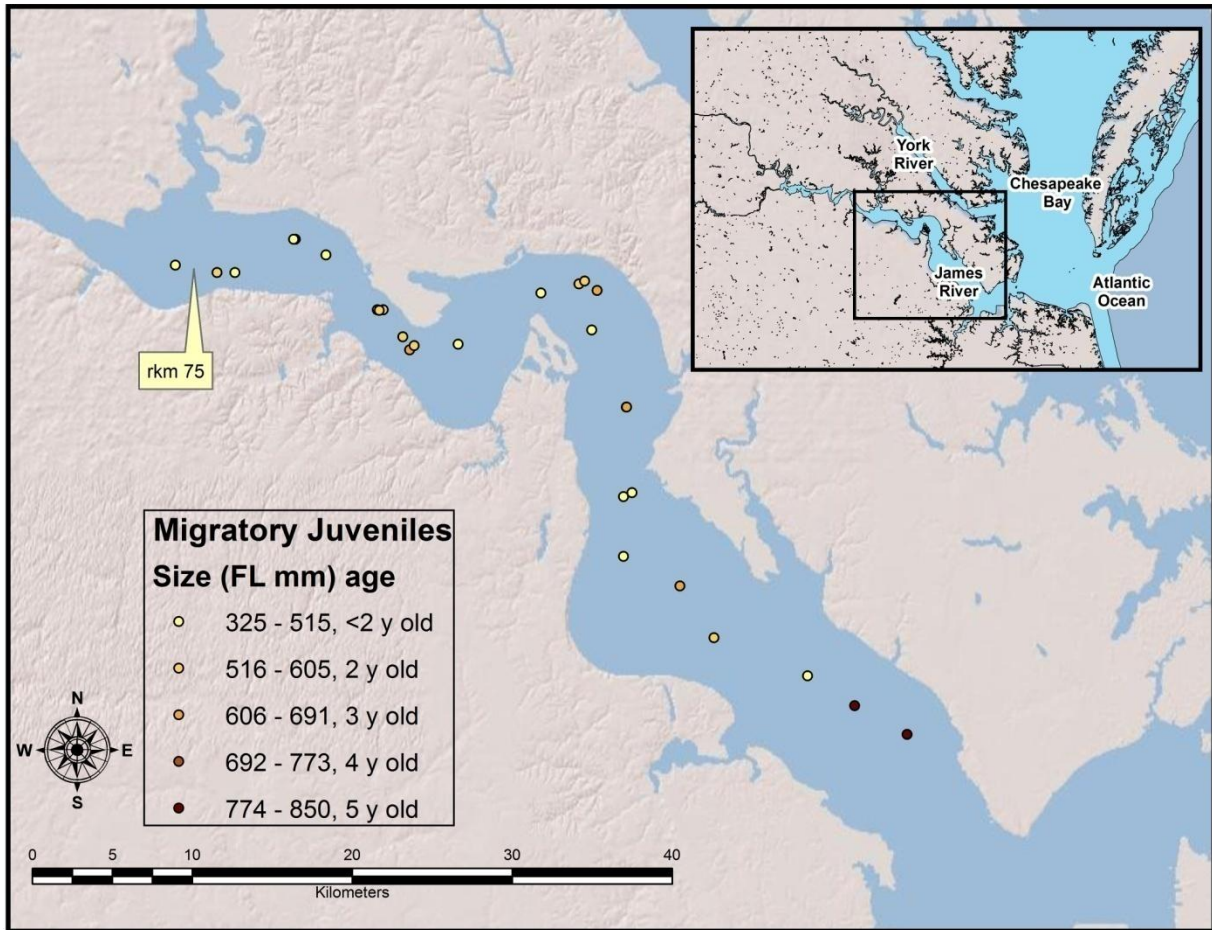


Figure 3. Map showing migratory juvenile collections made by the Virginia Institute of Marine Science trawl survey. Data points are color coded by fork length (mm).

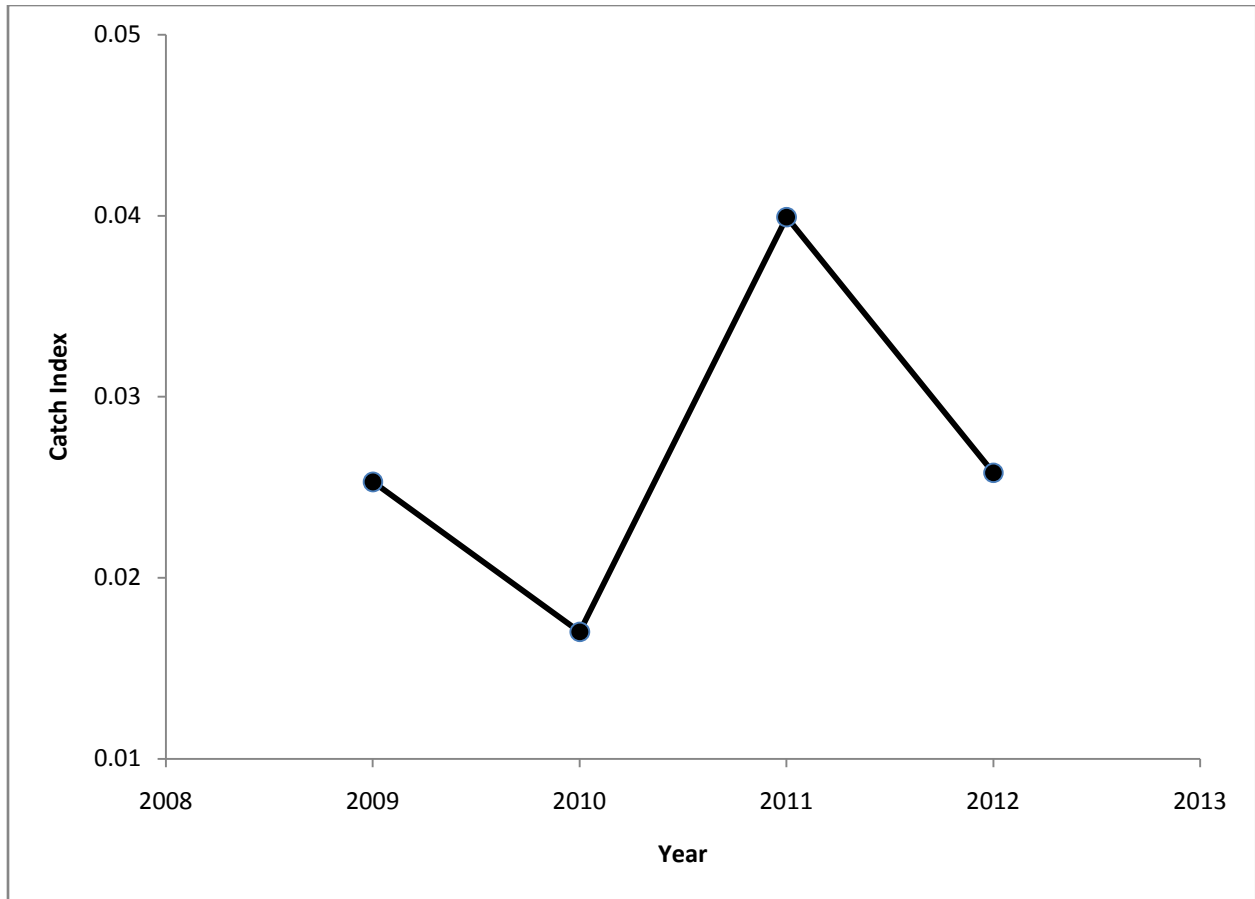


Figure 4. Catch index of adult Atlantic sturgeon. Catch index was determined using a general linear model with bias correction. Catch-per-unit-effort was fish caught per 100 m² of net fished per hour.

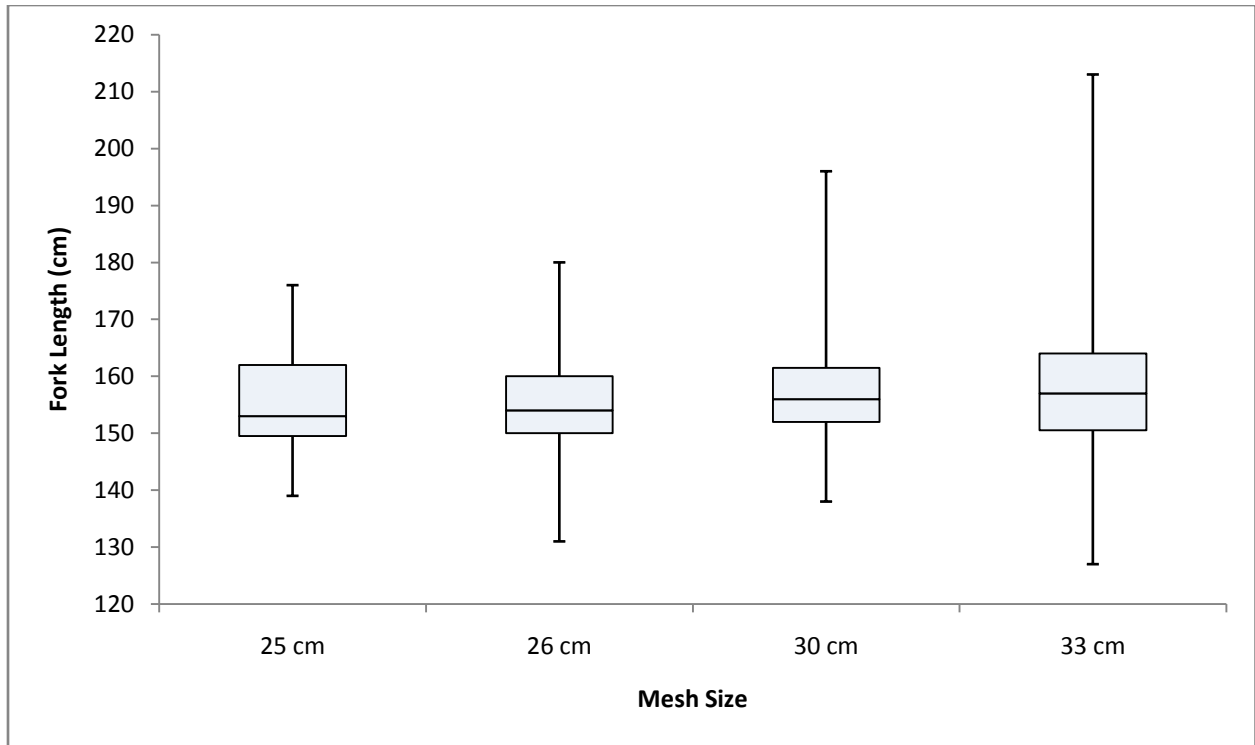


Figure 5. Boxplot showing catch distributions (fork length in cm) of various stretch mesh gill nets.

