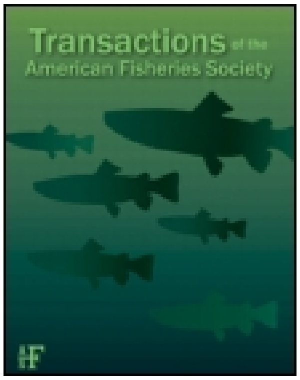

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Empirical Evidence of Fall Spawning by Atlantic Sturgeon in the James River, Virginia

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NOTE

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Abstract
Due to overfishing and habitat alteration, the anadromous Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* is severely depleted across its historic range. The James and York rivers in 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 river kilometer 108) was conducted in the spring (April–June) and fall (August–October) for 3 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 postspawned 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 the 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.

Due to overfishing and habitat alteration, populations of the anadromous Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* are severely depleted along its range from Labrador, Canada to Florida, United States (Hildebrand and Schroeder 1927; Smith 1985; Bain 1997; Boreman 1997). The National Oceanic and Atmospheric Administration 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 (NOAA 2012). 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 is important for future recovery efforts of this DPS to know if there are two spawning populations (spring and fall), which would allow for better management of river activities that might interfere with spawning adults and improve population estimates. Activities that are currently regulated include the timing of dredging in

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the James River, Virginia, to accommodate spring spawning of anadromous fishes. Perhaps dredging activities should also be regulated during the fall if fall-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 the reproduction of Mississippi River shovelnose sturgeon *Scaphirhynchus platorynchus* could be protracted into the fall or be 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 sturgeon (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. oxyrinchus 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 with 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 this study 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 to (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 (April–June) and fall (August–October). The National Oceanic and Atmospheric Administration 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 the 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 where 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 (°C) were recorded every day at gill-net sampling locations.

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

$$\frac{\text{Weight}}{\text{Fork Length}^3} \times 100{,}000,$$

where weight was measured in kilograms and FL in centimeters.

Gender was determined by our ability to manually express semen or eggs (Mohler 2003), 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 h 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 it from direct contact with the sample tubes, for overnight shipment to the University of Maryland Crane Aquaculture Facility.

On the day that a semen sample arrived at the Crane Aquaculture Facility, 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 μL of 20 mM tris-NaCl (80 mOsm/kg, pH 8.0) to 2 μL 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 ×. 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, 40 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). Tag range (1+ km) provided complete river width coverage in several areas of the river. The 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 tanker ships that operate consistently year round (Balazik et al. 2012).

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 1,232 net-hours in the spring and 949 net-hours in the fall. Effort in 2011 totaled 1,272 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 to 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°C to 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

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. The data for fork length, weight, girth, and condition factor are given as mean ± SD with the range in parentheses.

Measurement	2009	2010	2011
Hours of effort (spring/fall)	240/513	1,232/949	1,272/462
Spring dates of sampling	Apr 8 – Jun 5	Apr 11 – Jun 17	Apr 1 – Jun 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 7 – 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 the Atlantic sturgeon semen analysis in 2011. Fish identification number (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	Semen per sample (mL)	% Motility	Density ($\times 10^9$/mL)	mOsmol/kg
0118	Sep 13, 2011	Sep 15, 2011	25	60	2.4	122
3436	Sep 14, 2011	Sep 16, 2011	15	0	4.1	123
3141	Sep 14, 2011	Sep 16, 2011	13	50	2.5	122
0644	Sep 14, 2011	Sep 16, 2011	15	80	2.4	108
0964	Sep 14, 2011	Sep 16, 2011	13	30	1.2	131
2204	Sep 14, 2011	Sep 16, 2011	10	0	2.8	120
5313	Sep 21, 2011	Sep 22, 2011	25	80	3.9	135
1419	Sep 21, 2011	Sep 22, 2011	27	80	3.0	108
1932	Sep 21, 2011	Sep 22, 2011	9	90	2.8	110
3167	Sep 21, 2011	Sep 22, 2011	10	80	5.6	127
2918	Sep 21, 2011	Sep 22, 2011	15	50	3.9	150
4169	Oct 3, 2011	Oct 4, 2011	25	80	5.0	136
2918	Oct 4, 2011	Oct 5, 2011	25	0	4.8	203
2564	Oct 4, 2011	Oct 5, 2011	25	80	3.3	116
2525	Oct 4, 2011	Oct 5, 2011	15	50	7.1	234

2010 semen samples verified that activated semen was motile from all six males, for approximately 1 min. The more extensive examination of the 2011 samples showed variation in semen characteristics (Table 2). Sperm density varied from 1.2×10^9/mL to 7.1×10^9/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 postspawned female. The confirmed postspawned 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, had a girth of 76 cm, and a 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 and September 17 across all 3 years. One of these fish, captured on September 9, 2009, had a concave abdomen similar to the postspawn 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 (range, 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 5 fish in 2009, 25 fish in 2010, and 10 fish 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

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.

Measurement	2009	2010	2011
Number of tagged fish	5	25	10
Dates tagged	Aug 21–Sep 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–Jun 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+

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 d after arrival; however, four of them returned again in August along with another 11 adults tagged in 2009–2010. 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 data).

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, postovulated eggs recovered from the urogenital opening were in an early degradation stage, suggesting the fish had spawned within days (J. Van Eenennaam, University of California at 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 gill-net 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 within the range favorable for egg incubation (20–21°C) for Atlantic sturgeon and temperature of the Hudson River, New York when ripe broodstock are typically captured (23°C) (Mohler 2003).

When sexing sturgeon the 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 2 d postcollection. There was a possibility that the three samples with 0% sperm motility had been contaminated with water or urine. Three of the four fish of which sexing was attempted but could not be determined had higher condition factors, weights, lengths, and girths than 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 pigmented oocytes 1.5–1.7 mm in diameter (M. Balazik, 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 2003). 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. Nonreproductive 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 to locate specific spawning or habitat sites (Sulak and Randall 2002; Webb and Erickson 2007). The remaining nonsexed fish had a concave abdomen similar to the verified postovulatory female, and although no eggs were collected, the fish's morphology is consistent with that of a recently spawned female (Ryder 1890).

The 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 in the fall do not overwinter in the river to spawn later in the spring.

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 (13-15 cm FL) Atlantic sturgeon in the York River, December 2011–January 2012 (H. Brooks, Virginia Institute of Marine Science, personal communication). Using age-0 growth data for Delaware River Atlantic sturgeon, the York River age-0 fish were estimated to be 2.5–3.5 months posthatch (M. Fisher, Department of Natural Resources and Environmental Control-Division of Fish and Wildlife, personal communication), suggesting they were hatched in approximately the same time frame (September) as the captured postspawn 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.

If sexually mature fish are 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 a 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 U.S. 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 the following: deployment of egg collection mats, additional broodstock collection, sexing and staging maturity using ultrasound, gonad biopsy and plasma steroid analyses, continued acoustic tagging and tracking of adults, genetic analyses of spring- versus fall-captured adults, and additional collection and length-frequency evaluation of age-0 individuals.

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