Spawning Drivers and Frequency of Endangered Atlantic Sturgeon in the York River System

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[A] Abstract

There is limited information about Atlantic Sturgeon *Acipenser oxyrinchus oxyrinchus* spawning behavior despite over 100 years of commercial exploitation for their eggs. Spawning return intervals for males and females have been estimated in the most general of time spans while researchers only established in the last 25 years that sturgeon eggs and larvae are fresh water obligates, dispelling the notion that spawning occurred in estuaries. This study analyzes capture data from 2013 to 2019 to estimate Atlantic sturgeon spawning return intervals to the York River system, a tributary to the Chesapeake Bay in Virginia. Then, using female capture information, analyzes the abiotic influences that appear to drive egg deposition. Both males and females return to spawn at more frequent intervals than was reported in the literature with males returning once every 1.13 years and females returning once every 2.19 years. Three females were documented returning to spawn in consecutive years; one of them returning five out of six years.

All females captured on the spawning grounds were gravid with eggs at stage 5 or further progressed. In all years, 105 fall adult females were caught; 73 were stage 5, 26 stage 6, and 6 stage 7. Of the 26 stage 6 females, 13 were actively releasing eggs when captured. Egg deposition is correlated with photoperiod, water temperature, and a drop in barometric pressure in the 24 hours prior to capture. Ten of 13 females releasing eggs were caught during day lengths within 20 minutes of the autumn equinox. Females releasing eggs were only captured at water temperatures between 21.5 °C and 25.1 °C. This information should provide the foundation of predictive models that allow researchers and managers to understand how these endangered species are likely to respond to climate change.

[A] Introduction

Atlantic Sturgeon *Acipenser oxyrinchus oxyrinchus* are long-lived, late maturing, iteroparous, anadromous fish (Smith 1985; Bemis and Kynard 1997; Dadswell 2006; NMFS 2007; Peterson et al. 2008). Sexual maturity varies latitudinally with males maturing slightly younger than females in all systems and fish in southern systems maturing as early as 4 years, while taking as long as 27 years in northern systems (Scott and Crossman 1973; Peterson et al. 2008). Sexual maturity appears more size-dependent than age-dependent (Caron et al. 2002). Spawning locations must be sufficiently far above the saltwater interface to allow larvae to drift downstream while remaining in freshwater (Bain1997; Markin 2017).

The interval between spawning events has been reported broadly to span as many as five years with females having more non-spawning years than males (Smith 1985; Bain 1997; Caron et al. 2002; Peterson et al. 2008; Dadswell et al. 2017; Taylor and Litvak 2017). Within these broad ranges, northern populations skip spawning more frequently than southern populations (Hilton et al. 2016). Managers may interpret all upstream migrations as being motivated by spawning, however sub-adults will also move into freshwater reaches for obviously non-sexual reasons (Hager 2016). ASMFC (2017) and Kahn et al. (2019) have proposed metrics for confirming spawning sturgeon populations. Van Eenennaam and Doroshov (1998) and Collins et al. (2000) provide detailed descriptions of the female egg maturation process, where some egg stages can be used to confirm spawning in that system.

Atlantic Sturgeon spawn in deep river sections (> 7.6 m; Bain 1997; Caron et al. 2002), often in remote upriver regions, making it difficult to know when and where spawning is occurring or if it is occurring at all. Because of this, NMFS (2007) suspects Atlantic Sturgeon historically spawned in 35 major rivers. Today it is estimated that between 19 and 27 river systems still support spawning (Hilton et al. 2016; ASMFC 2017). Much of what has been published on Atlantic Sturgeon reproductive behavior was derived from fisheries dependent data at a time when sturgeon were thought to reproduce in estuaries (Dovel and Berggren 1983; Smith et al. 1984). We now know sturgeon in estuaries are a composition of mixed populations (Waldman et al. 1996; Wirgin et al. 2015), confounding previous conclusions about spawning behavior or abundance (Smith 1985; Secor 2002; Kahnle et al. 2007).

Relying on fisheries dependent data, all Atlantic Sturgeon were previously believed to spawn in the spring (Smith 1985). As fisheries independent data has been collected, Atlantic Sturgeon spawning behaviors appear highly varied, with a number of different reproductive strategies employed along the Atlantic Coast. Recent research has documented fall spawning from Georgia to Virginia (Collins et al. 2000; Balazik et al. 2012; Hager et al. 2014; Smith et al. 2015; Ingram and Peterson 2016). Spring spawning occurs north of the Chesapeake Bay (Bain 1997; Hatin et al. 2002; Dadswell et al. 2017, Taylor and Litvak 2017). Dual spawning has been confirmed in the Edisto River in South Carolina (Collins et al. 2000). More recently, it was suggested that all rivers supported dual spawning (Balazik and Musick 2015), which was not ultimately supported with data (Kahn et al. 2019) but it is possible, yet still unconfirmed, that dual spawning takes place in the James River, Virginia, the southernmost tributary of the Chesapeake Bay (Balazik and Musick 2015).

Atlantic sturgeon spawning is generally discussed in a broad range from 13 °C to 26 °C that could support spawning from Canada to Georgia (Smith 1985; Kahnle et al. 1998; Peterson et al. 2000; Caron et al. 2002; Hatin et al. 2007; Wippelhauser et al. 2017). In some rivers, spawning temperatures have been produced. Spawning typically occurs between 18 °C and 20 °C in the St. John (Taylor and Litvak 2017; Mitchill et al. 2020). Larvae were captured in the Kennebec River when water temperatures were between 23 °C and 24 °C (Wippelhauser et al. 2017). Peak spawning occurs in the Hudson River when temperatures are between 13 °C and 18 °C (Kahnle et al. 1998). Post-spawn females were documented in the York and James Rivers between 24 °C and 25 °C (Balazik et al. 2012; Hager et al. 2014). Back-calculated spawning temperatures derived from captured larvae in the Roanoke River suggest spawning occurs between 24 °C and 25 °C (Smith et al. 2015). The Edisto River supports spawning in the spring and fall with documented spawning temperatures between 13 °C and 14 °C in March and 17 °C to 18 °C in September and October (Collins et al. 2000).

This study used seasonal sampling to determine when spawning occurred and telemetry detections to address Atlantic Sturgeon spawning return intervals. We were able to combine sexual stage of telemetered males and females with tracking data to conclude spawning return intervals. Opportunistic captures of endangered adult Atlantic sturgeon during the spawning season were also correlated with a number of abiotic conditions to infer the factors that induce spawning of females.

[A] Methods

[C] *Study area*. – The York River, Virginia, is located along the western edge of the Chesapeake Bay, north of the James River, south of the Rappahannock River (Figure 1). The York River is formed by the confluence of the Pamunkey River, 150 km long, and the Mattaponi River, 166 km long. The York River is 55 km long and ranges from oligohaline at the confluence of its two main tributaries in West Point, Virginia, to polyhaline at its mouth just east of Gloucester Point, Virginia. Atlantic Sturgeon have been confirmed spawning in the Pamunkey River (Hager et al. 2014) and young of year sturgeon have been incidentally captured in the lower Mattaponi River where it meets the Pamunkey River (Tuckey and Fabrizio 2012), however the confluence is low salinity and allows movement of young of year between the two. The York River has no freshwater reaches and does not support spawning of Atlantic Sturgeon. Though most of the length of both the Mattaponi and Pamunkey Rivers are spring-fed and tidal freshwater, their lower reaches are oligohaline.

[C] *Collection methods*. – Sampling occurred at river kilometer 74 from late July through mid-October each year between 2013 and 2019 (see Kahn et al. 2019). Atlantic Sturgeon sampling was conducted in the spring and fall but the York River system only supports fall spawning (Kahn et al. 2019). Gill nets were custom made, ranging in size from 23 to 36 cm stretch mesh, 91 m long but anchored on each bank, sized to extend from surface to river bottom, and set three in sequence in a 0.35 km section of river. Because sturgeon are endangered, many were cut out of the nets resulting in many nets used over the seven seasons; the shortest was 6.5 m tall, fished in 4.9 m of water and the tallest was 7.3 m tall, fished in 6.7 m of water. The three nets were always different mesh sizes to have an equal likelihood of catching adult sturgeon of all sizes with the largest mesh downstream and smallest mesh in the middle. Temperatures from late July to October ranged from 30.3 to 16.7 °C, but sampling was restricted to times when temperatures were below 28 °C (Kahn and Mohead 2010).

[C] *Sex determination and staging*. – Individuals were sexed during surgery or by applying pressure to the ventral surface moving from the anterior to posterior ends, ending at the vent. Van Eenennaam and Doroshov (1998) and Collins et al. (2000) produced clear descriptions of sexual stages of males and females. Males were non-invasively sexed as "milting" or remained sexually unidentified. Female egg stage was defined by Van Eenennaam and Doroshov (1998) and the stages observed during this study are:

Stage 5: migratory nucleus. The ovarian folds are filled with fully grown (diameter 2.61 ± 0.12 mm), darkly pigmented oocytes possessing a thick three-layered envelope with micropyles. The germinal vesicle is displaced to the animal pole and the oocyte has a distinct polarized structure, with a higher concentration of large yolk platelets and oil droplets in the vegetal hemisphere.

Stage 6: oocyte maturation. Ovulated (or approaching ovulation) oocytes have undergone germinal vesicle breakdown and nuclear material is mixed with the cytoplasm.

Stage 7: postovulatory. The ovaries contain numerous empty postovulatory follicles.

Only these final three stages are explicitly defined or referenced because this study is specific to a freshwater location approximately 39 km upstream of the saltwater interface during the spawning season; no earlier egg stages were observed. Stage 6 is discussed later and specifically identified either as above or as two phases: eggs released from the ovaries and loose in the body but not being released and eggs actively being released. Most commonly, gravid females did not produce gametes with pressure but were confirmed female when transmitters were implanted and egg samples were taken at that time. One or two eggs were removed from each female for staging. Female reproductive tissue was not biopsied, so when no eggs were found, either because the fish was spent or male, sex remained unidentified. Sex was occasionally confirmed upon recapture.

[C] *Calculation of spawning return frequency and detections*. – To calculate return rates of males and females between 2014 and 2019, we relied on telemetry data. Between 2013 and 2017, 54 acoustic transmitters (Vemco V16P-4H, V16P-6x, or V16-6x) were implanted in sturgeon. Incisions were 2 to 4 cm in length, made most often between the 3rd and 4th ventral scutes anterior to the anal fins, into which a transmitter was inserted. The incisions were closed using Vicryl® dissolvable sutures. After surgery was complete, fish were released approximately 1.5 km from the capture site to avoid multiple captures in one day. If a fish was captured twice in the same day (n = 47), nets were removed for the rest of the day to avoid harassing fish attempting to use the sampling location. The deployed transmitters were programmed to transmit a 69 kilohertz (kHz) signal every 70 to 150 seconds and had a life span of at least 6 years. When tags ceased being detected, they were no longer used to estimate return frequencies.

The implanted transmitters were passively tracked within the freshwater and saline reaches of the York River system year-round from 2014 through 2019. Figure 1 shows passive Vemco VR2W-69 kHz receiver stations within the York River and the Chesapeake Bay. Seventy receivers maintained by Chesapeake Scientific and the US Department of the Navy remained in place for the duration of this study. From July to November, additional receivers were placed in the Mattaponi and Pamunkey rivers. Because of the narrow width (most locations < 25 m) of the Pamunkey and Mattaponi rivers, the receivers acted as gates where every fish that passed a receiver would be within a detectable range, verified through range studies presented by Hager

(2016). Receivers were placed near the surface, faced downward, and were serviced and downloaded monthly.

Atlantic Sturgeon were determined to be on a spawning run if they moved at least 20 km upriver of the saltwater interface (Van Eenennaam et al. 1996; Kynard and Horgan 2002) and spent at least 17 consecutive days in freshwater based on ad-hoc observations. Relying on the detections of individuals returning to spawning grounds each year, we used a ratio estimator:

$$\bar{p}_r = \frac{\Sigma\, T_{return}}{\Sigma\, T(t)}$$

where $\bar{p}_r$ is the mean proportion of telemetered fish (of each sex separately) to return over the duration of the estimate, while T_{return} and $T_{(t)}$ represent the total number of individuals of each sex that returned to spawn in each year and the total number of individuals of each sex that could have returned to spawn in those years, respectively. When transmitters were lost or failed, they were removed from this estimate following their final detection. Likewise, fish that were detected in another river system were not counted in T_{return} and $T_{(t)}$ for that year. The Vysochanskij-Petunin inequality (Vysochanskij and Petunin 1989; Andrushkiw et al. 2005) was used to calculate 95% confidence limits (CLs) of the return intervals.

[C] Calculation of factors contributing to egg deposition. – The correlation between abiotic factors and female reproductive stage were made qualitatively. For this analysis, we recorded the date of capture, photoperiod (sunrise to sunset), water temperature at the sampling location, egg stage of females captured, fork length (FL), flow rate, 24 hour change in flow rate, 24 hour proportional change in flow rate, barometric pressure, and 24, 48, 72, and 96 hour temperature and pressure changes before capture. To understand the factors associated with spawning, we primarily considered stage 6 females, with a real focus on those females releasing eggs at the time of capture.

When possible, abiotic conditions were collected onsite, but when measurements could not be made at the sampling location, we relied on the nearest location. All captured sturgeon were measured to the nearest millimeter FL. Water temperatures were recorded using a HOBO Onset U12-015 temperature logger tethered to the river bank and suspended at 1.5 m depth at low tide. While temperatures were recorded every 3 hours, we relied on the midday temperature measurement for that day. Sunrise and sunset times corresponding to each sampling date were downloaded from the website "timeanddate.com" using the location: Pamunkey Indian Reservation (approximately 17 km east). Flow data was obtained from the U.S. Geological Survey gage 01673000, from the Pamunkey River near Hanover, Virginia (approximately 20 km west). Barometric pressure was obtained from the National Oceanic and Atmospheric Administration, National Centers for Environmental Information, from Doswell, Virginia (approximately 24 km west).

[A] Results

Between 2013 and 2019, 283 male and 105 female Atlantic sturgeon were captured during the fall spawning season; many of those captured multiple times during the study. Of the 105 adult females, 73 were stage 5, 26 stage 6, and 6 stage 7 (Figure 2). Of the stage 6 females, 13 had released eggs into their abdominal cavities without physically expressing eggs, while 13 were captured releasing eggs; in the act of spawning. All six stage 7 females expressed eggs with pressure, but in 66% of those cases, the eggs were grey and non-viable.

Spawning frequency was estimated using telemetry tagged males (n = 28) and females (n = 26). When calculating spawning return interval in the season following transmitter implantation, males returned 115 times out of a possible 130 spawning events between 2013 through 2019 (Table 1). Females returned 36 times of a possible 79 spawning events. The mean spawning return rate for males was every 1.13 years (95% CL, 1.12 – 1.14) with most males returning every year and only one male skipping two consecutive years. Females returned on average every 2.19 years (95% CL, 1.92 – 2.56) with most spawning every other year, occasionally in consecutive years, and only one female skipped three consecutive years (Table 1).

Actively spawning females (n = 13) provide the greatest insight into the conditions that result in spawning at the sampling site. There was no consistent correlation between fish length, time of capture, or the tidal conditions and capture during egg release (Table 2). Actively spawning females were always captured near the lead line of the gill net. Two females releasing eggs were captured at the downstream end of the deepest pool in the area (6.5 m) and most spawning fish were captured in water over 3 m measured from the location captured and not the deepest location sampled by the net that captured them.

Three of the abiotic drivers considered were correlated with female egg release. First, temperature appears to be an important influence on egg release. No females were captured releasing eggs outside of the thermal window ranging from 21.5 ℃ to 25.1 ℃ (Table 2). Photoperiod is also closely correlated with female egg stage. Ten of the 13 (77%) females releasing eggs were captured within 30 minutes of the autumn equinox. The majority of stage 6 and 7 females (13 of 19, 68.4%) were captured when day length was between 12 hours and 12 hours, 20 minutes with a peak when sunrise was 06:56 and sunset was 19:00. Finally, for 70% (22 of 32) of the females in stage 6 or 7, 77% (10 of 13) of females releasing eggs, and 79% (15 of 19) of females releasing eggs or spent, the barometric pressure had dropped in the 24 hours prior to their capture by an average of -1.422, -2.133, and -2.404 millibars, respectively. There was no apparent correlation with female egg stage and fork length (FL), flow rate, 24-hour change in flow rate, 24-hour proportional change in flow rate, barometric pressure, and any other temporal changes in temperature or barometric pressure.

[A] Discussion

Atlantic Sturgeon spawning in the York River occurs in the fall as water temperatures decline from summer highs through optimal bioenergetic temperatures (Niklitschek 2001). Sturgeon move upriver between July and August and there is a closed spawning population through most of September (Kahn et al. 2019). The females in this study were captured throughout the fall spawning period and every captured female was gravid with eggs staged as 5, 6, or 7. Similar developmental distribution was reported in the Hudson River with females being stage 5 or later when upriver (Van Eenennaam et al. 1996).

Analysis of telemetry data from 2014 through 2019 reveals a narrower spawning return interval with more frequent spawning events than reported by others in more northern systems (Bain 1997; Billard and Lecointre 2001; Dadswell 2006). York River males return to spawn every one to three years, with only one fish ever skipping two seasons, while females return to spawn every one to four years, occasionally returning in consecutive years. In this study, the variables of T_{return} and $T_{(t)}$ were calculated from the year following transmitter implantation. Because transmitters were implanted in Atlantic sturgeon while they were on spawning runs, those values could have been calculated to include the year of implantation. As a result, the reported spawning return frequencies may slightly under-estimate the true rate. Female return rates, who usually skip a year between spawning events, are most likely under-estimated because our calculations begin counting their possible returns in the year we would expect them to remain at sea. In one exceptional instance, female 14-034 was present on spawning grounds during five out of six possible spawning events. She was captured on two of those returns and was stage 5 each time. Female 14-054 was also captured in 2014, 2015, and 2017 in stage 5 each year. To our knowledge, these are the first documented Atlantic sturgeon females confirmed to be in gravid condition on spawning grounds in consecutive years. In captive White Sturgeon *A. transmontanus*, it is possible for a female with stage 5 eggs to retain those eggs until the following spawning season (Joel Van Eenennaam, UC Davis, personal communication), so this may not be confirmation of a female spawning in consecutive years.

Female spawning condition changes relatively rapidly in the Pamunkey River. In 2019, female 17-041 was captured on September 18 and 23. On September 18, her eggs were stage 5 and only 5 days later she had lost over 27.2 kg of eggs, continued to express eggs with pressure, and still appeared roughly ¼ full (Table 2). There is also a close temporal relationship between females in stage 6 and stage 7, often caught in the same year at roughly the same time. For this reason, it is interesting that 4 of the 6 stage 7 females had grey non-viable eggs. This suggests the transition from viable, spawning eggs to non-viable post-spawn eggs may be very rapid, in some cases occurring while the female is still on the spawning grounds and prior to outmigration.

Spawning events are poorly documented for Atlantic sturgeon; typically limited to evidence of a single spawning event (Collins et al. 2000; Smith et al. 2015). During this study, it appears temperature, photoperiod, and a drop in barometric pressure in the 24 hours prior to spawning are important drivers for egg release. Nineteen adult females were captured either releasing eggs or spent, of which 13 were captured when photoperiod was between 12.3 and 12.0 hours. Research on other sturgeons has shown photoperiod is also the primary driver for spawning in different systems and at different times of years (Papoulias et al. 2011; Kieffer and Kynard 2012). Likewise, spawning for many other teleost fish has been linked to photoperiod (Norberg et al. 2004; Migaud et al. 2006), and of potential importance for sturgeon caviar production, artificially manipulating photoperiod has been shown to increase reproductive rates (Whitehead et al. 1978; Carrillo et al. 1989; Campos-Mendoza et al. 2004).

Water temperature during the fall spawning season (16.7 ℃ to 30 ℃) is closely correlated with photoperiod but varies considerably between years. The 32 individuals who were stage 6 or 7 in this study were all captured within the same 0.35 kilometer stretch of river at temperatures between 20.6 ºC and 25.5 ℃ (Figure 2). Twenty-six of those were caught in water cooler than 25 ℃, two actively releasing eggs at 25.1 ℃, and four with eggs released from the ovarian folds but not being expressed at temperatures ranging from 25.1 to 25.5 ℃. The 13 females captured while actively releasing eggs were at temperatures between 21.5 ℃ and 25.1 ℃ (Table 2). Temperatures above 25 ℃ are associated with high sturgeon egg mortality (Chapman and Carr 1995; Ingram and Peterson 2016). Thermal windows essential for spawning have been observed for Shortnose Sturgeon *Acipenser brevirostrum* as well (Kieffer and Kynard 2012).

The narrow temperature range when sturgeon were observed spawning correlates with observed optimal growth rates for age-1 Hudson River Atlantic Sturgeon (Niklitschek 2001) and may provide more insight into the reasons for egg deposition regardless of spring or fall spawning (Markin and Secor 2020). Niklitschek (2001) showed an optimal growth combination of temperature and dissolved oxygen (DO) for juvenile Atlantic Sturgeon of roughly 18 to 22 ℃ at 70 to 100 percent DO saturation, which is the typical DO saturation in the Pamunkey River during spawning season. The data collected in this system suggest females release their eggs in the temperature range between the upper threshold for optimal egg survival (Chapman and Carr 1995) and upper threshold for optimal juvenile growth (Niklitschek 2001). The drop in barometric pressure observed for every group of stage 6 and 7 combinations may indicate timing is also influenced by the onset of fall storms that would cause water temperature to drop. In that way, female Atlantic sturgeon may be able to release their eggs near the upper thermal limits for eggs and increase the likelihood of high survival.

Atlantic Sturgeon appear to time their spawning to maximize larval growth through the entire range of optimal temperatures. Baird et al. (2019) showed juvenile green sturgeon predation risk from Largemouth Bass *Micropterus salmoides* and Striped Bass *Morone saxatilis* decreased with growth until nearing zero at 200 to 220 mm total length. Both of these predators are present in the Pamunkey River and increasing growth rates at the most vulnerable size may increase the likelihood of year class success. Further research will be necessary to understand the predation threat from these species in this system as well as from the introduction of the invasive Blue Catfish *Ictalurus furcatus*.

Identifying the parameters that drive spawning in a single system provides managers with justification for protective mitigation within that system. Improving our understanding of the factors that drive and thus may limit the species reproductive potential provides data crucial to conservation of the entire species. The data presented here could be used to develop predictive models of Atlantic Sturgeon spawning given shifts in local weather patterns due to climate change. Between 2013 and 2019, the preferred photoperiod for spawning did not fluctuate even though the proposed thermal window for spawning expanded by approximately 2 days each year, thus extending the potential spawning window by two weeks by 2019. Additional research is needed to address population variability of Atlantic Sturgeon genetics, bioenergetics, system-specific life histories, seasonality of egg release, and seasonally available larval food resources to understand how the species is likely to respond to climate change.

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Table 1. Telemetered fish identification numbers, sex, and seasons when a spawning run was made (X). Lengths and weights are reported from their initial capture. When an individual was believed dead, a “-” was used to note that no spawning run could be made and a “†” represents evidence of spawning in a different river. Not all rivers have receiver arrays, so it is also possible some of these individuals spawned in unmonitored systems without being detected.

Fish ID	FL (mm)	Weight (Kg)	Sex	2013	2014	2015	2016	2017	2018	2019
14-012	1995	84.7	Female		X	-	-	-	-	-
14-023	1773	56.8	Female		X		X		X	
14-034	1988	83.6	Female		X	X	X		X	X
14-037	1813	61.0	Female		X		X		X	
14-054	1790	58.6	Female		X	X		X		X
15-003	1820	61.8	Female					X		X
15-010	1935	76.1	Female			X		X		X
15-011	1899	71.3	Female			X			X	
15-020	1855	65.8	Female					X		X
15-035	1845	64.7	Female			X		X		X
15-048	2188	119.9	Female			X		X		X

16-008	1984	83.1	Female				X		X	
16-009	1873	68.0	Female				X		X	
16-010	1592	41.1	Female				X			
16-013	1867	67.3	Female				X		X	
16-020	1984	83.1	Female				X		X	
16-023	2038	91.6	Female				X		X	X
16-027	1881	69.0	Female				X		X	
17-003	1663	46.6	Female					X		X
17-011	2065	96.2	Female					X		X
17-016	1978	79.6	Female					X		X
17-019	1636	46.6	Female					X		X
17-031	1890	70.1	Female					X		X
17-033	1738	54.1	Female					X		X
17-036	1858	66.2	Female					X		X
17-041	2150	117.3	Female					X		X
17-053	2004	86.1	Female					X		X
13-002	1652	34.3	Male	X	X	X	X	X	X	X
13-003	1503	30.7	Male	X	X	X	X	X	X	X
13-004	1541	32.5	Male	X	X	X	X	X	X	X
13-005	1298	20.8	Male	X		X	X	X	X	X
13-007	1490	33.3	Male		X	X	X	X	X	X
13-009	1382	24.9	Male	X	X	X	X	X	X	X
13-012	1585	37.6	Male	X	X	X		X	X	X
13-013	1653	37.9	Male	X	X	X	X	X	X	X
13-015	1548	28.8	Male	X	X	X	X	X	X	X
14-002	1593	34.0	Male		X		X	X	X	X
14-004	1489	30.0	Male		X	X	X	X	X	X
14-007	1502	30.7	Male		X	X	X	X	X	X
14-008	1514	30.1	Male		X	X		X	X	X
14-009	1572	34.0	Male		X		†		†	
14-013	1624	36.6	Male		X	X	X	X	X	X

14-015	1533	32.2	Male			X	X	X	X
14-020	1481	30.0	Male	X	X		X	X	X
14-024	1499	30.5	Male	X	X	X	X	X	X
14-026	1709	40.7	Male	X	X	X	X	X	X
14-028	1528	28.7	Male	X	X	X	X	X	X
14-029	1367	24.9	Male	X	X	X	X		X
14-031	1540	32.5	Male	X	X	X	X	-	-
14-032	1666	38.6	Male	X	X	X		-	-
14-036	1659	38.2	Male	X			X	X	X
14-043	1634	42.9	Male	X	X		X		X
14-050	1679	39.2	Male	X	-	-	-	-	-
16-039	1647	37.7	Male			X	X		†
16-042	1452	28.3	Male			X	X	X	X

Table 2. Physical description of females actively releasing eggs (late stage 6) along with relevant biotic and abiotic information.

ID	Fork length	Date	Time	Temp (ºC)	Tide	Slack tide	Estimated progression	Estimated depth (m)	River morphology
14-033	1880	9/12/14	1415	24.5	Flood	1500	½ spent	4 to 5	Mainstem, confluence with large creek
15-048	2225	9/22/15	0830	22.7	Flood	1120	Initiating, full of eggs	6.5	Center river, deep hole
15-029	1867	9/30/15	1115	22.8	Ebb	1300	¾ spent	6.5	Center river, deep hole

16-009	1921	9/6/16	1340	23.5	Ebb	1500	½ spent	4 to 5	Mainstem, confluence with large creek
16-010	1609	9/6/16	1625	23.5	Flood	1500	½ spent	3 to 4	Mainstem, confluence with small creek
14-037	1958	9/22/16	1700	22.8	Slack	1630	Initiating, full of eggs	3 to 4	Straight, thalweg
16-013	1915	9/29/16	1145	21.5	Flood	1100	½ to ¾ spent	3 to 4	Mainstem, confluence with large creek
17-031	1930	9/25/17	1140	23.5	Flood	0910	½ spent	3 to 4	Mainstem, confluence with large creek
18-009	1900	9/17/18	1230	25.1	Slack	1200	½ spent	2 to 3	Beside pool, along dropoff
18-010	2051	9/17/18	1450	25.1	Ebb	1800	½ spent	2 to 3	North bank along dropoff
19-016	1833	9/21/19	0900	21.7	Slack	915	¼ spent	3	Upstream of small creek
17-041	2200	9/23/19	1125	23.7	Slack	1155	¾ spent	3 to 4	Mainstem, confluence with large creek

19-043	2074	10/3/19	850	24.8	Slack	840	¾ spent	2 to 3	South bank along dropoff

Figure 1. The York River and its tributaries the Pamunkey and Mattaponi rivers. Dots along the map represent telemetry receivers and the star represents the upstream sampling station where females were collected.

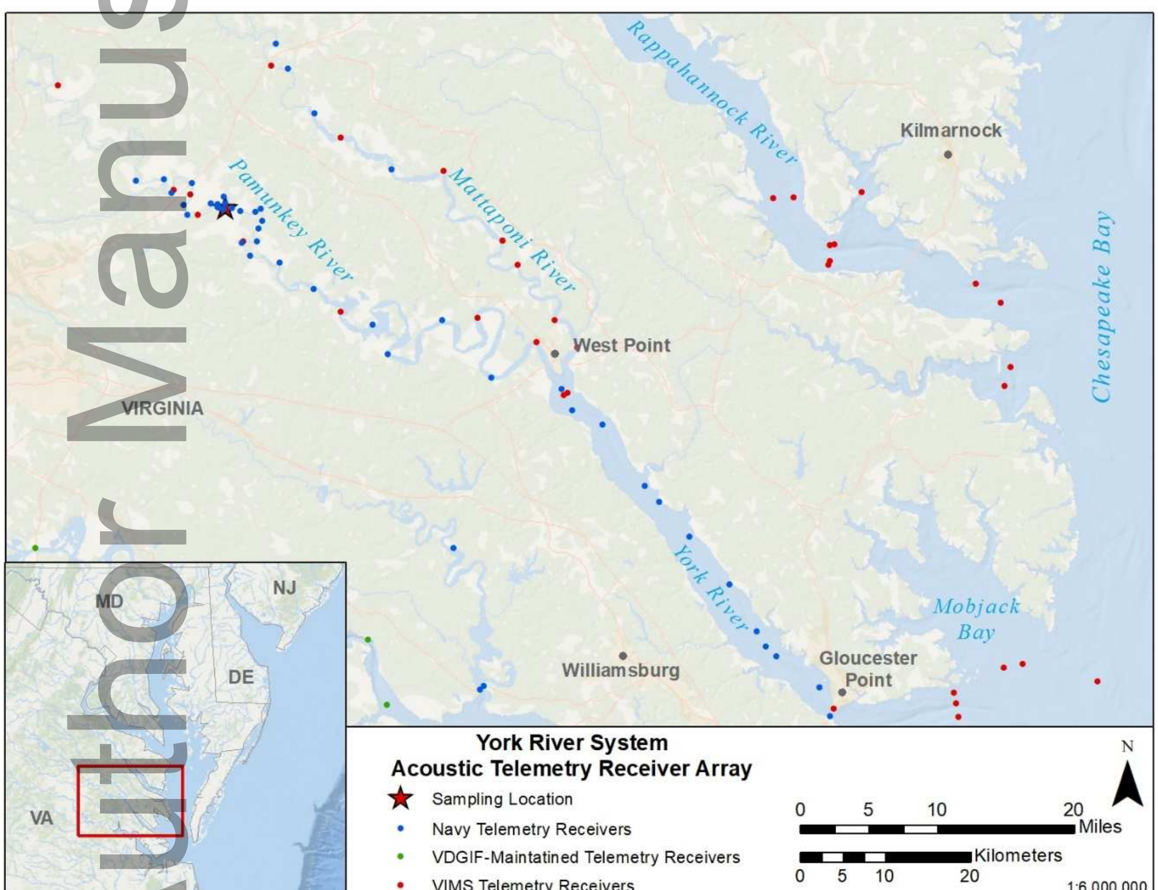
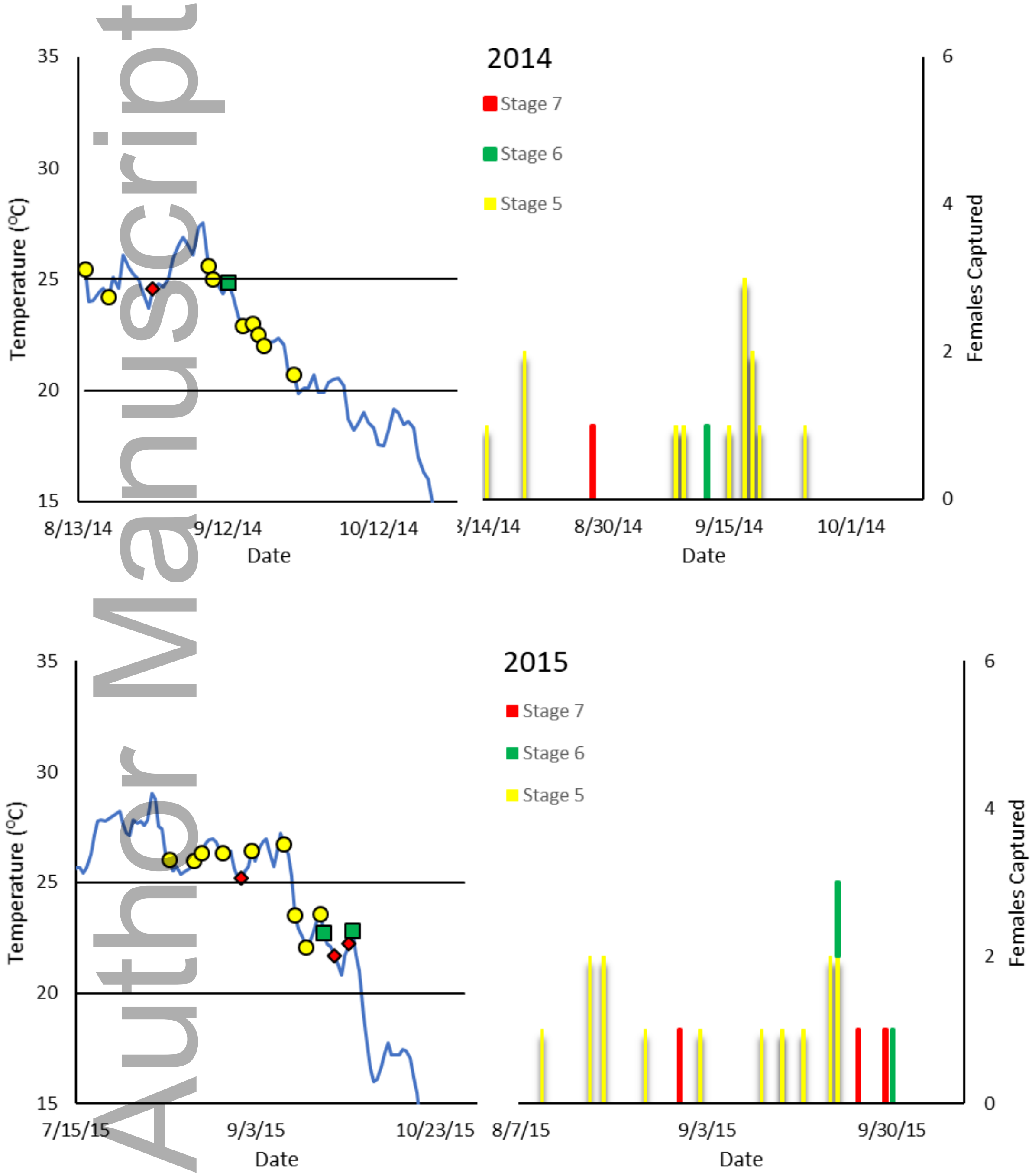
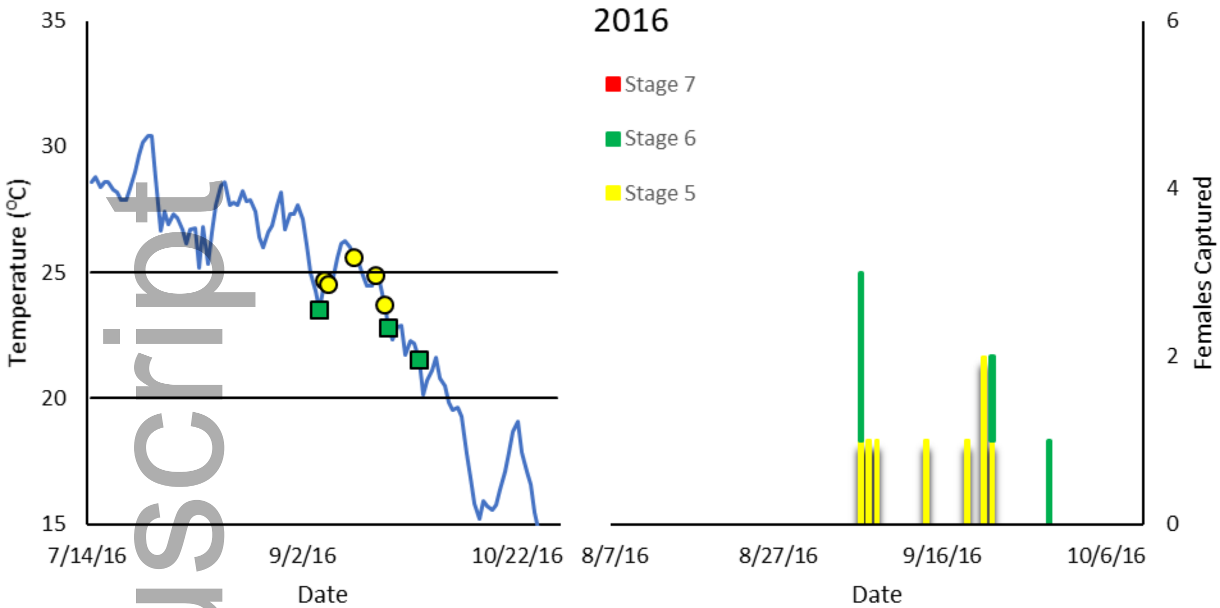
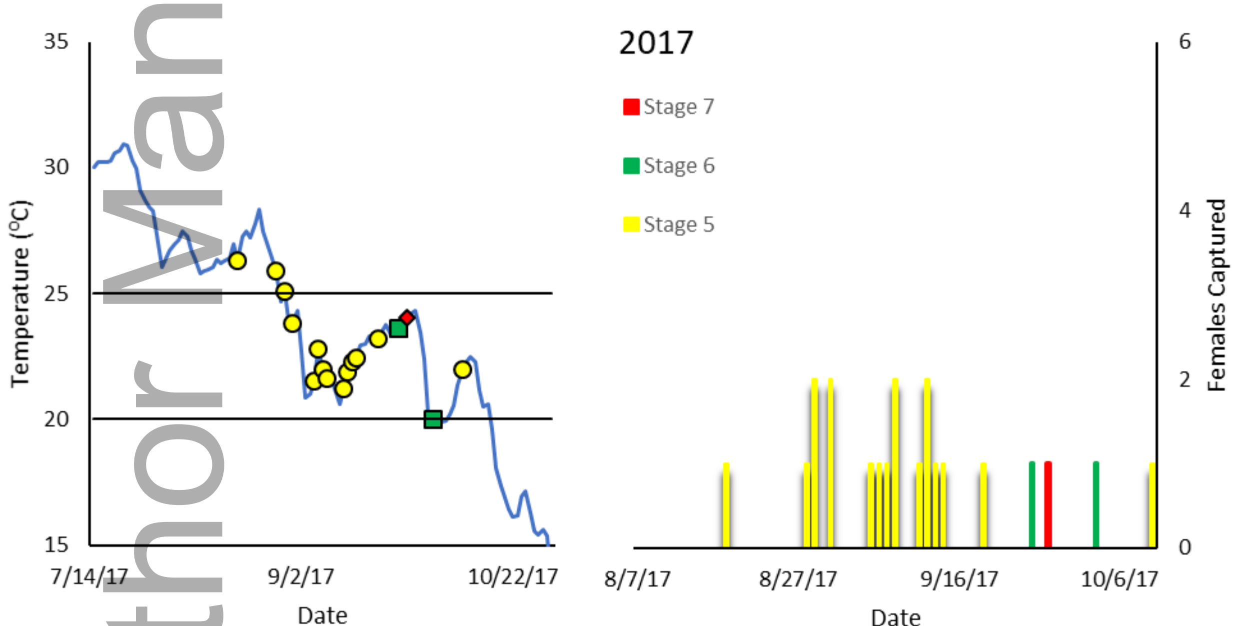
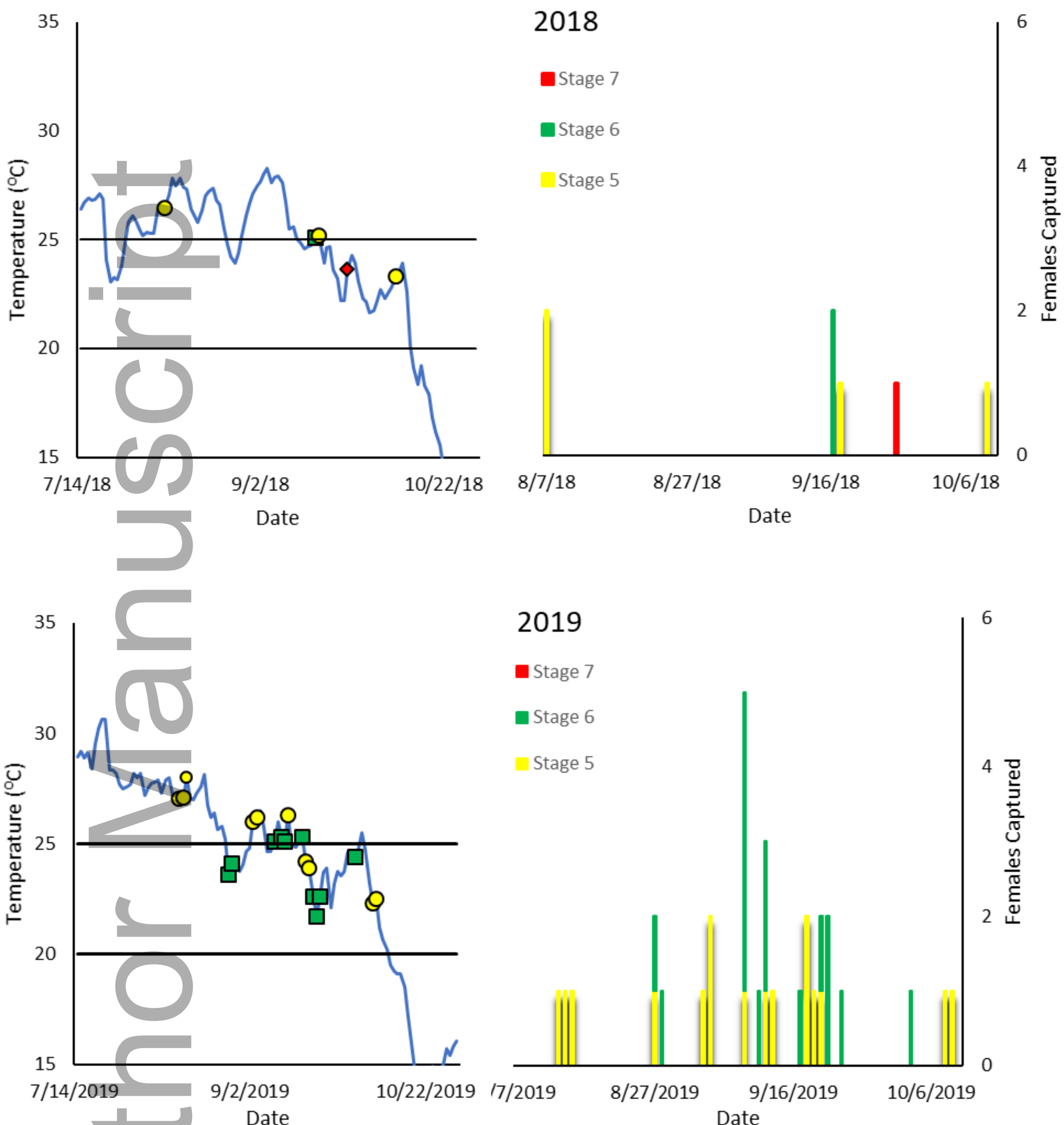


Figure 2. Correlation between females captured each year from 2014 to 2019 and the declining temperature from summer to fall. The lines at 25°C and 20°C depict the temperatures where spawning appears to be initiated and terminate




2016
Stage 7
Stage 6
Stage 5
Temperature (°C)
35
30
25
20
15
7/14/16
9/2/16
10/22/16
Date
8/7/16
8/27/16
9/16/16
10/6/16
Date
Females Captured
6
4
2
0


2017
Stage 7
Stage 6
Stage 5
Temperature (°C)
35
30
25
20
15
7/14/17
9/2/17
10/22/17
Date
8/7/17
8/27/17
9/16/17
10/6/17
Date
Females Captured
6
4
2
0


2018
Stage 7
Stage 6
Stage 5
Temperature (ºC)
Females Captured
35
30
25
20
15
6
4
2
0
7/14/18
9/2/18
10/22/18
Date
8/7/18
8/27/18
9/16/18
10/6/18
Date
2019
Stage 7
Stage 6
Stage 5
Temperature (ºC)
Females Captured
7/14/2019
9/2/2019
10/22/2019
Date
/7/2019
8/27/2019
9/16/2019
10/6/2019
Date