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ORIGINAL ARTICLE

Development and testing of an environmental DNA (eDNA) assay for endangered Atlantic sturgeon to assess its potential as a monitoring and management tool

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Abstract

Significant declines in Atlantic sturgeon (*Acipenser oxyrhynchus oxyrhynchus*) abundances along the US east coast have spurred major research efforts and management actions over the last 50 years, yet information on spawning stock abundances and habitat use is still lacking for many river systems, including in the Chesapeake Bay, USA. Here, we developed and tested a new quantitative PCR (qPCR) assay to detect Atlantic sturgeon environmental DNA (eDNA) in water samples with the goal of providing an alternative method to monitor presence and relative abundance. We also examined Atlantic sturgeon eDNA shedding rates in laboratory experiments. A qPCR-probe assay targeting Cytochrome B was developed and showed no amplification of other related and co-occurring fishes. Pond trials at a density of ~0.2 g/L sturgeon produced relatively strong eDNA detections (~1,000–25,000 copies/L) in all seven water samples assayed. Water samples taken from two river systems in the Chesapeake Bay produced zero eDNA detections in the summer, while fall sampling during sturgeon spawning produced positive eDNA detections in 26% of samples, though at much lower concentrations (400–1,800 copies/L) compared with the pond (mesocosm) detections. Acoustic detections of sturgeon near river sampling sites were positively associated with eDNA detections. However, the eDNA assay failed to detect the presence of sturgeon in some samples when abundances were very low or when fish were in deep water. Finally, Atlantic sturgeon eDNA shedding rates were estimated to be on the order of estimates for other fish species, which suggests that relatively weak detections in the field are not necessarily driven by low rates of eDNA shedding. Overall, eDNA analysis represents a promising new monitoring tool for Atlantic sturgeon. Applying these methods in other rivers along the US east coast is an important next step in documenting Atlantic sturgeon distribution for management and conservation purposes.

KEYWORDS
conservation, decay, fish, spawning, telemetry, tidal river

1 | INTRODUCTION

Atlantic sturgeon (*Acipenser oxyrhinchus oxyrhinchus*) are long-lived, anadromous fish that reside in large rivers along the US East Coast, with populations ranging from the St. Johns River in Florida to Labrador Canada (Smith & Clungston, 1997). Sturgeon once supported major fisheries across many Atlantic coastal rivers, but by the early 1900s, most populations had declined significantly due to overfishing and abundances have remained low ever since (Secor & Waldman, 1999; Smith, 1985; Waldman & Wirgin, 1998). Human-mediated changes to critical estuarine and riverine habitats, such as river channelization, dam construction, vessel strikes, and water quality degradation, have further reduced population abundances over the last century (Smith, 1985; Waldman & Wirgin, 1998). Atlantic sturgeon are a long lived, but late maturing species (15–20 years for females; Gilbert, 1989), with significant intervals between successive spawns, so they are likely to have slow population recovery. The species also exhibits relatively low gene flow among populations and high return rate to natal rivers for spawning (King et al., 2001; O'Leary et al., 2014; Wirgin et al. 2000), which can make stocks particularly vulnerable to localized changes in habitat quality or pollution.

Twenty-seven rivers support spawning populations of Atlantic sturgeon on the US East Coast (ASSRT, 2007; Hilton et al. 2016), which have been grouped into five distinct population segments (DPS) based on genetic data (e.g., ASSRT, 2007; Grunwald et al. 2008; Hilton et al. 2016; King et al. 2001; O'Leary et al., 2014; Wirgin et al. 2000). Four of these DPS (New York Bight, Chesapeake Bay, Carolina, South Atlantic) are listed as endangered under the ESA (Hilton et al. 2016; NMFS, 2012a, b). All populations have experienced significant declines in abundances compared to historical levels, but spawning stock abundances within each DPS vary, and for many rivers, population status is uncertain (ASSRT, 2007; Hilton et al. 2016; NMFS, 2012a, b). Information on the Chesapeake Bay DPS is particularly lacking, but three spawning populations are known to exist currently: the James River, the Pamunkey River of the York River system, and the Nanticoke River system (Hilton et al. 2016; NMFS, 2012a, b). Despite some estimates of adult spawning numbers on the James (Balazik et al. 2012; Balazik & Musick, 2015), and Pamunkey rivers (Hager et al. 2014; Kahn et al. 2014), in general, population trends for the Chesapeake Bay spawning subpopulations are lacking.

Environmental DNA (eDNA) has emerged as a powerful approach to survey the occupancy and relative abundance of fishes and other aquatic animals from residual DNA left behind in the aquatic environment (Jerde et al. 2011; Lacoursière-Roussel et al., 2016; Lodge et al. 2012; Thomsen, Kielgast, Iversen, Moller, et al., 2012; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012). High assay sensitivity (detections down to a few DNA copies) coupled with simple sampling and processing protocols (~0.5–3 liters sampled from surface waters), makes this approach especially useful for rare and elusive taxa at low abundance (e.g., Atlantic sturgeon). eDNA analysis is also much less labor-intensive compared to traditional sampling methods, such as electrofishing or net-based capture, which require specialized boats, equipment, sampling permit authorizations, and relatively large teams that can only sample a limited number of rivers in a workday. eDNA sampling can be performed individually from shore, potentially covering multiple sampling locations within a short spawning season (e.g., Plough et al. 2018). eDNA surveillance is less invasive than capture approaches that can damage tissues or harm fish during collection (e.g., Bohl et al. 2009; Jordan et al. 2008). The use of eDNA to survey fish populations has become increasingly common over the past decade, with studies conducted on a variety of species across a range of freshwater and marine environments (e.g., Doi et al. 2017; Plough et al. 2018; Takahara et al. 2012; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012). A novel eDNA assay was developed for threatened Gulf sturgeon (*Acipenser oxyrhynchus desotoi)* and the extremely rare and endangered Alabama sturgeon (*Scaphirhynchus suttkusi)* in the Mobile River basin (Pfleger et al. 2016). Field sampling produced positive identifications of both species, with higher detection probability in spring/summer during seasonal spawning migrations. Given that Atlantic sturgeon are in higher abundances than the Alabama sturgeon (at least in some rivers), the development of a novel eDNA assay to detect Atlantic sturgeon should be possible and would greatly benefit management by providing an alternative method to characterize the temporal and spatial distribution of the species within understudied river systems.

In this study, our objective was to develop and test a probe-based qPCR assay for detecting Atlantic sturgeon eDNA in water samples from a mesocosm (pond) and water samples from two river systems in the Chesapeake Bay. River samples were collected proximal to passive acoustic telemetry receivers during and outside of sturgeon spawning periods to allow comparison of acoustic detections of tagged Atlantic sturgeon in these systems to eDNA detections and relative abundance. We also quantified Atlantic sturgeon eDNA shedding rates in mesocosm experiments to better understand potential constraints on the detection of eDNA in the environment. This work builds upon previous efforts to develop eDNA resources for Atlantic sturgeon in the James River, Chesapeake Bay by Hinkle (2015).

2 | MATERIALS AND METHODS

2.1 | Assay design

Multi-species sequence alignments were created from publicly available sequence data in the National Center for Biotechnology Information's (NCBI) Genbank repository (http://www.ncbi.nih.gov/genbank), from which potential primers and probe regions were identified. Three mitochondrial genes with substantial, multi-species sequence resources were targeted for 5' nuclease probe development: cytochrome oxidase 1 (CO1), NADH dehydrogenase subunit 2 (ND2), and cytochrome B (CytB). Alignments were created with all nine species and (or) subspecies of sturgeon in North America including the genus *Acipenser*, the genus *Scaphirhynchus*, and one species of paddlefish (*Polyodon spathula*). Sequences from other commonly

co-occurring fishes in the Chesapeake Bay watershed were included in the alignment to check for sufficient mismatches to more distantly related species, including Atlantic menhaden (*Brevoortia tyrannus*), striped bass (*Morone saxatilis*), longnose gar (*Lepisosteus osseus*), and alewife (*Alosa pseudoharengus*). Probe regions were chosen for maximal numbers of mismatches to nontarget species (≥3), but no within-species mismatches (e.g., no single nucleotide differences among Atlantic sturgeon sequences retrieved for the alignment). Primer/probe sets were also tested for potential off-target complementarity with PrimerBlast (Ye et al. 2012) following their design.

Two probe assays were designed per gene using the OligoArchitect Online software (Sigma Aldrich). Assays were tested initially on fin clip DNA from eight Atlantic sturgeon from the Altamaha River, Georgia, to examine basic amplification performance (e.g., size of amplicons, PCR efficiencies, and primer dimer). Probes and primer sets were ordered as PrimeTime® qPCR 5′ nuclease probes from Integrated DNA Technologies (IDT; Coralville Iowa), in a double-quenched orientation with FAM as the reporter dye. qPCRs were performed in 20 µl volumes using 10 µl of 2× SSO Advanced Universal Probe Mix (BioRad, Hercules CA), 1 µl of Forward and Reverse primers (10 µM), 0.6 µl of 10 µM concentration fluorescent probe primer, 10 µl water, and 4 µl of DNA (~15–20 ng total). qPCR reactions were run on the BioRad CFX 96 qPCR machine at a range of different annealing temperatures (52°C–62°C) in the following protocol: 1 min denaturation at 95°C, then 40 cycles of 10 s at 95°C, 30 s at 52–62°C, and 10 s at 72°C, with a plate read after the extension step (72°C) in each cycle. Of the six assays tested, one of the two CytB probe assays performed the best, demonstrating strong amplification efficiency with no background (limited primer dimer). This probe/primer set amplified a 144bp stretch of the CytB gene with an optimal annealing temperature of 60°C (Table 1) and was pursued further for testing against nontarget fin clip DNA. As shown in the alignment (Appendix S1, File A1), numerous mismatches were present for other fishes in the probe region for this assay, and there were at least three mismatches to all North American sturgeon and paddlefish species except for Gulf sturgeon. Additional mismatches for nontarget fish and sturgeon were present in the F and R primer sites as well. Probe/primer sets could not be designed to discriminate Gulf sturgeon (*Acipenser oxyrhynchus desotoi*), which showed no sequence differences across ~600 base pairs (bp) of CytB, only a single difference (one single nucleotide polymorphism or SNP) across ~700 bp of CO1, and only four differences (single SNPs spaced 50+ bp apart) across ~500 bp of ND2.

2.2 | Testing the CytB qPCR assay in vitro with fin clip DNA

The CytB primer/probe set was tested in qPCR reactions with fin clip DNA from a range of co-occurring Chesapeake Bay fishes, including Atlantic menhaden (*B. tyrannus*), river herring (*Alosa pseudoharengus* and *Alosa aestivalis*), bay anchovy (*Anchoa mitchilli*), striped bass (*M. saxatilis*), gizzard shad (*Dorosoma cepedianum*), and shortnose sturgeon (*Acipenser brevirostrum*) to test assay specificity. qPCR assays were run in duplicate on DNA samples from two different fish for each species (four qPCR reactions run per fish species) alongside Atlantic sturgeon fin clip DNA. To test assay efficiency and determine initial gene copy number in unknown fin clip and environment samples, we used a 143 bp synthesized fragment (oligo) of the CytB gene (IDT gBlocks gene fragment) from published Atlantic sturgeon sequence (GenBank accn: KU985073.1) as a standard in qPCR runs. The synthesized CytB fragment was serially diluted based on mass from 300,000 to 30 copies to create a standard curve and was run in duplicate in all qPCR runs of environmental samples to facilitate calculation of the starting copy numbers in each fin clip or unknown eDNA sample (see below). For initial testing and comparison of amplification in other fishes, synthesized standards were run in triplicate. Cycle threshold (Cq) value, the PCR cycle at which fluorescence rises above background, was determined for each sample (standards and unknowns) with the BioRad CFX Manager software (v3.1), using a single-point threshold (100 RFU) and the baseline substitution curve fit for drift correction. PCR inhibition was tested by spiking the 30,000 or 300,000 copy standard with 2 µl of DNA from each DNA sample to ensure that sample amplification was not prevented due to inhibitory compounds in the extraction (e.g., Plough et al. 2018). To relate Cq value to mtDNA copy number (to determine eDNA abundance from a given sample), Cq was regressed against (known) Log copy number for each of the standards from 30 to 300,000, and unknown samples were fit to this curve. qPCR runs with regression $r^2 < 0.98$ were repeated. PCR reactions were performed in low-profile BioRad white (opaque) skirted 96 well plates and sealed with Biorad "Adhesive B" seals.

TABLE 1 Primer, probe, and qPCR information for the CytB assay

Primer Name	Sequence	qPCR cycle conditions
ATS-CytB-Reverse	CGGTTATTATGGTGAGAAG	(1) 95°C for 1 min
		(2) 95°C for 10 s
ATS-CytB-Forward	CCGAAATATTCATGCAAAC	(3) 60°C for 30 s
		(4) 72°C for 10 s (plate read)
ATS-CytB-PROBE[a]	/56FAM/CCTCCTTCT/ZEN/ TCTTCATTTGCCTGT/3IABkFQ/	(5) Go to Step 2 for 39 cycles

[a]Primers and probes ordered as PrimeTime® qPCR 5′ nuclease probes from Integrated DNA technologies (IDT) with 5′ reporter dye FAM and two quenchers, ZEN and IowaBlack.

2.2.1 | Pond mesocosm sampling

Our mesocosm was a large earthen pond with a surface area of 0.13 ha, average depth of 1.8 m, and approximate volume of 2,336 m^3. This pond contained 46 Atlantic sturgeon with a total biomass of 443 kg that were introduced four months prior to this experiment—no other fish species were introduced or present, to our knowledge. Water samples (1–2 liters) were collected by hand at the water surface from a 1.2 m long dock at three time points from November 2016 to February 2017.

2.2.2 | Field sampling

We sampled two tidal rivers on opposite shores of the Chesapeake Bay: the Pamunkey River (a tributary of the York River) in Virginia, the Nanticoke River on Maryland's Eastern Shore, and Marshyhope Creek, a tributary of the Nanticoke River (Figure 1). In each river, we collected 1–2 liters of water by hand at the water surface from boats. A total of 17 water samples were collected from the Pamunkey River at six locations in 2016 on October 3, 12, and 20 (see Table 2 for site details). In the Nanticoke River or Marshyhope Creek, 12 samples were taken on

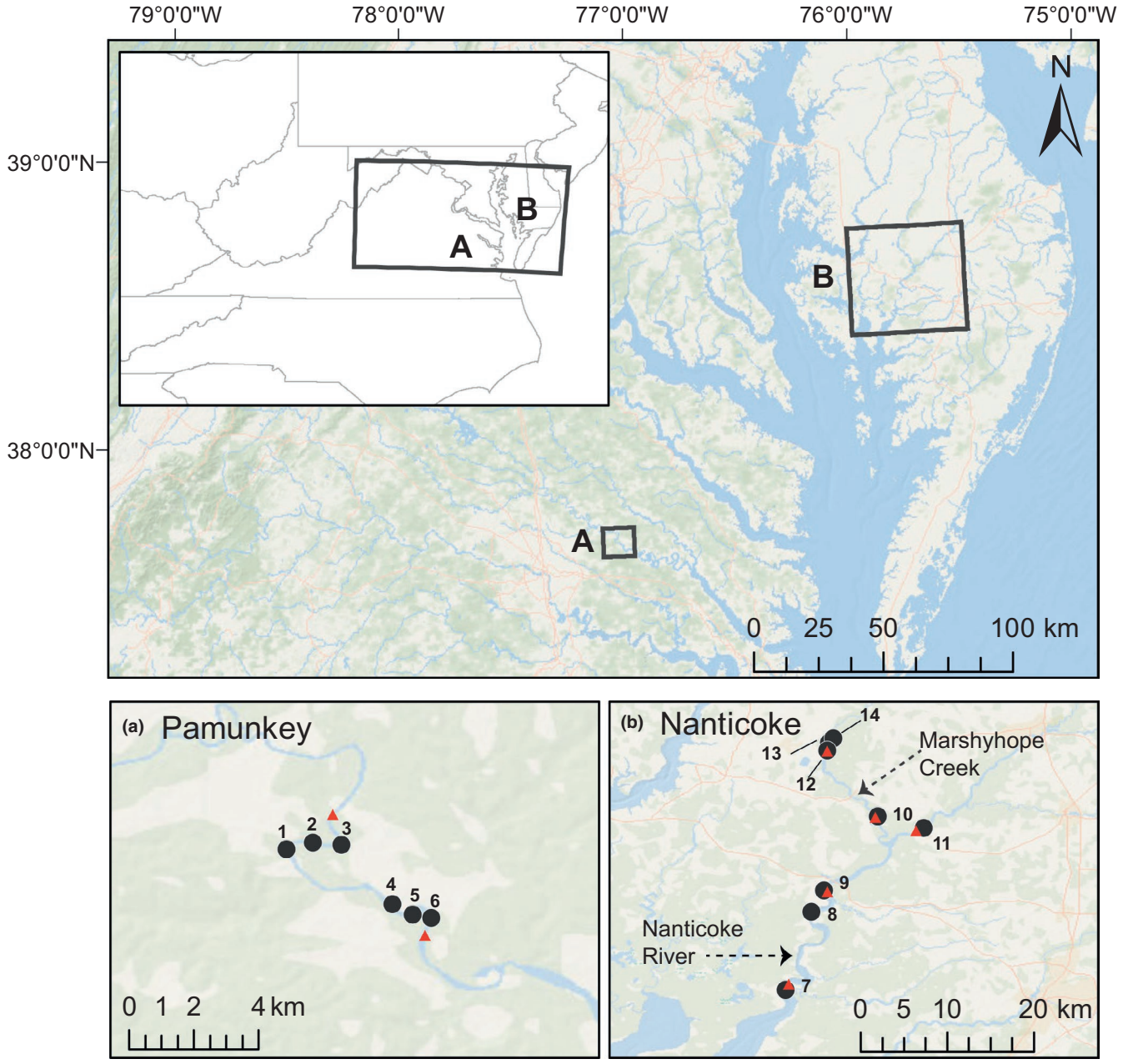

FIGURE 1 Map of sample sites (filled black circles) and acoustic telemetry receivers (filled red triangles) on the Pamunkey River (Panel A) and Nanticoke River or Marshyhope Creek (Panel B) in the Chesapeake Bay region, USA. Numbers next to sample sites (1–14) correspond to the site number in parentheses in the "Site" column in Table 2. Longitude and latitude are shown at the top and left side of the regional (upper) map, respectively

TABLE 2 eDNA sampling sites and results

River	Site[a]	Date	Latitude	Longitude	Copies/L	Acoustic detection[b]
Pond	Pond	11/29/16	NA	NA	981	NA
Pond	Pond	11/29/16	NA	NA	25,342	NA
Pond	Pond	12/16/16	NA	NA	9,398	NA
Pond	Pond	12/16/16	NA	NA	11,567	NA
Pond	Pond	12/16/16	NA	NA	22,263	NA
Pond	Pond	2/22/17	NA	NA	3,485	NA
Pond	Pond	2/22/17	NA	NA	9,806	NA
Marshyhope Cr.	392 Overpass (13)	7/14/16	38.6325	−75.8176	0	0
Marshyhope Cr.	Ab. Walnut land. (10)	7/14/16	38.5561	−75.7673	0	0
Marshyhope Cr.	Ab. Walnut land. (10)	7/14/16	38.5561	−75.7673	0	0
Nanticoke	Butler Beach (8)	7/14/16	38.4571	−75.836	0	NA
Nanticoke	Butler Beach (8)	7/14/16	38.4571	−75.836	0	NA
Nanticoke	Chapter pt. (7)	7/14/16	38.3765	−75.863	0	1
Nanticoke	Chapter pt. (7)	7/14/16	38.3765	−75.863	0	1
Nanticoke	Control	7/14/16	NA	NA	0	NA
Nanticoke	Ferry Pt Vienna (9)	7/14/16	38.4796	−75.8232	0	1
Nanticoke	Ferry Pt Vienna (9)	7/14/16	38.4796	−75.8232	0	1
Nanticoke	Sharptown (11)	7/14/16	38.5437	−75.7214	0	0
Nanticoke	Sharptown (11)	7/14/16	38.5437	−75.7214	0	0
Marshyhope Cr.	392 overpass (13)	9/21/16	38.6325	−75.8176	1817	1
Marshyhope Cr.	Ab. 392 overpass (14)	9/21/16	38.6375	−75.8131	0	1
Marshyhope Cr.	Ab. 392 overpass (14)	9/21/16	38.6375	−75.8131	649	1
Marshyhope Cr.	Bel. 392 overpass (12)	9/21/16	38.6246	−75.8193	0	1
Nanticoke	Ferry Pt Vienna (9)	9/21/16	38.4796	−75.8232	0	0
Nanticoke	Control	9/21/16	NA	NA	0	NA
Pamunkey	Chericoke-lower (1)	10/3/16	37.6351	−77.1296	834	1
Pamunkey	Chericoke-middle (2)	10/3/16	37.637	−77.1223	494	1
Pamunkey	Chericoke-upper (3)	10/3/16	37.6364	−77.1145	796	1
Pamunkey	Control	10/3/16	NA	NA	0	NA
Pamunkey	Putney's-lower (6)	10/3/16	37.6161	−77.0895	0	1
Pamunkey	Putney's-middle (5)	10/3/16	37.617	−77.0947	0	1
Pamunkey	Putney's-upper (4)	10/3/16	37.6199	−77.1002	0	1
Pamunkey	Chericoke-lower (1)	10/12/16	37.6351	−77.1296	0	1
Pamunkey	Chericoke-middle (2)	10/12/16	37.637	−77.1223	0	1
Pamunkey	Chericoke-upper (3)	10/12/16	37.6364	−77.1145	0	1
Pamunkey	Control	10/12/16	NA	NA	0	NA
Pamunkey	Putney'-middle (5)	10/12/16	37.617	−77.0947	563	1
Pamunkey	Putney's-lower (6)	10/12/16	37.6161	−77.0895	0	1
Pamunkey	Putney's-upper (4)	10/12/16	37.6199	−77.1002	0	1
Pamunkey	Chericoke-lower (1)	10/20/16	37.6351	−77.1296	0	1
Pamunkey	Chericoke-middle (2)	10/20/16	37.637	−77.1223	0	1
Pamunkey	Chericoke-upper (3)	10/20/16	37.6364	−77.1145	0	1
Pamunkey	Control	10/20/16	NA	NA	0	NA
Pamunkey	Putney's-lower (6)	10/20/16	37.6161	−77.0895	0	1

(Continues)

TABLE 2 Continued

River	Site[a]	Date	Latitude	Longitude	Copies/L	Acoustic detection[b]
Pamunkey	Putney's-middle (5)	10/20/16	37.617	−77.0947	0	1
Pamunkey	Putney's-upper (4)	10/20/16	37.6199	−77.1002	0	1

[a]In site names, "Above" is abbreviated as "Ab.", "Below" is abbreviated as "Bel.", and "Landing" is abbreviated as "Land.". Numbers in parentheses at the end of site names correspond to the site numbers in Figure 1. Only one passive acoustic receiver was proximal to any given site (392 overpass, Chericoke, Putney's, Sharptown, Chapter Pt, Ferry Pt, Above Walnut landing), but Butler Beach had no proximal acoustic receivers.; [b]Acoustic data are presented here as "present" (1) if there were any detections of tagged sturgeon within the five days prior to eDNA sampling or "absent" (0) if there were no detections (see Figure 3 for quantitative acoustic data for particular sites/days). Pond samples had no acoustic data and acoustic data for eDNA controls (cooler blanks) were not presented (not relevant).

July 14, 2016 when sturgeon were not spawning and expected to be in very low abundance. Subsequent water samples were collected on 21 September 2016 (n = 5) when sturgeon were expected to be in higher abundances during spawning. This was validated with passive acoustic receiver data for tagged Atlantic sturgeon (see below). To test for potential sample contamination during water collections, we filled sterile bottles with deionized (DI) water, submerged them in river water, and then placed them in a cooler on each sampling day ("cooler blanks" or controls). One control sample was taken each sampling day for each river (Nanticoke/Marshyhope or Pamunkey) at a randomly chosen site (five control samples in total; Table 2).

2.2.3 | Sample preparation

Water samples from both pond mesocosm and river samples were frozen at −20°C until filtering in the fall of 2017. Water samples were filtered on 47 mm diameter, 1.0 micron (pore size) cellulose nitrate (CN) filters (Whatman) in a dedicated tissue and water processing, pre-PCR laboratory. Filters for each water sample (up to 3) were stored at −80°C in individual 15 ml Falcon tubes until extraction with the EZNA water kit (Omega Biotek, Norcross GA). All freezers were monitored in real time for temperature via the Sensaphone Web600, which sends an alarm signal if freezer temperature rises above a set threshold. The −20°C freezers (where water samples were stored) never went above −15°C during water storage, and the −80°C freezer (where filters were stored) never went above −65°C. Each extraction was then run through a Zymo Research OneStep PCR Inhibitor Removal column (Irvine CA) to remove any residual inhibitory compounds. All permits required for handling and culturing of Atlantic sturgeon, as well as permits required for sampling and transfer of tissue from Shortnose and Atlantic sturgeon for genetic analysis, were obtained for this work (NMFS Permit Numbers 20,314, 21,198 and 21,434). Research protocols have been approved by the Institutional Animal Care and Use Committee (IACUC) under protocol F-HPL-17-11.

2.2.4 | qPCR of eDNA samples

Quantitative PCR (qPCR) for eDNA samples was performed similarly to the fin clip DNA tests (see above) with a few minor differences. qPCR reactions for each unknown sample were run in triplicate, alongside the synthesized Atlantic sturgeon CytB oligo standard curve (300 to 300,000 copies), and each reaction contained 4 µl of extracted DNA in 20 µl volumes, with 10 µl of 2× Sso Universal Probe Supermix (BioRad, Hercules CA), 0.6 µl of the ATSCytB-F primer, 0.6 µl of the ATSCytB-R primer, and 0.6 µl of the ATSCytB-Probe (each at a concentration of 10 µM), and 4.2 µl of molecular biology-grade water. Cycle conditions were identical to those described above, with a 60°C annealing temperature. Cycle threshold (Cq) values of 39 or lower in at least two of three replicates were considered as positive detections of Atlantic sturgeon eDNA, and abundance of each sample was calculated based on the standard curve and reported as copies per Liter of filtered water. All positive eDNA detections were then re-amplified with the forward and reverse primers and PCR products were submitted to the Arizona State University DNA laboratory for Sanger sequencing to confirm target species amplification. For sequencing at the ASU DNA laboratory, PCR products were magnetic bead-purified and cycle-sequenced via the Applied Biosystems Big Dye V3.1 chemistry and samples run on an Applied Biosystems 3730XL Sequence Analysis Instrument.

2.2.5 | Acoustic data

Two stationary acoustic receivers (VEMCO® VR2W) were placed in close proximity (within 1 km) to upstream (Chericoke) and downstream (Putney's) sampling sites on the Pamunkey River from August-October 2016 (Figure 1). The use of Atlantic sturgeon detection data from sturgeon tagged during U.S. Navy-funded studies was granted for the purposes of showing the number of tagged individuals at the time of water collections.

Passive acoustic receivers were deployed on the Nanticoke River (n = 8) and the Marshyhope Creek (n = 15) from March-December 2016 on private piers, United States Coast Guard (USCG) assets, and attached to dedicated buoys where no other attachments were available. Data from five of these receivers were utilized for this study, all of which were within 1 km of eDNA collection sites (Figure 1). One receiver was proximal to each of the eDNA collection sights, with the exception of Butler Beach (Table 2, Figure 1).

2.2.6 | Statistical analysis of eDNA versus acoustic data

To evaluate the quantitative relationship between acoustic and eDNA detections of Atlantic sturgeon, we examined various regression models. Acoustic data for a given site were summarized as the cumulative count of unique, tagged sturgeon detected over a given time period prior to the eDNA sample collection, either two days prior to eDNA sampling (sum of detections 0, −1, −2 days from eDNA collection) or five days prior to eDNA sampling (sum of detections 0, −1, −2, −3, −4, −5 days from eDNA collection). Linear regression and nonparametric correlation analysis (Spearman's Rho) were performed to examine the relationship between the cumulative number of sturgeon detected within two or five days of eDNA sampling versus eDNA abundance (copies/L). Logistic regression was performed to examine the relationship between acoustic detections (cumulative counts of sturgeon two or five days prior to eDNA collection) and eDNA detection as a binary variable (eDNA present or absent). Overall model significance for each acoustic predictor variable (2 or 5 day cumulative counts) for the logistic regressions was approximated with chi-square tests. All statistical analyses were performed in R (R Core Team, 2018).

2.2.7 | eDNA shedding rate experiment

Shedding rate experiments were performed in the spring of 2019 (March-April) in two, 2.4 m diameter fiberglass tanks with filled volumes of 2,900–3,100 L at depths of 0.6 to 0.65 m. Tanks were arranged next to each other with a few inches between them. Prior to shedding rate experiments, unfiltered flow-through water from the Choptank River (6–10 ppt salinity, 10–14°C) was provided at a rate of ~50 L per minute. Fish were kept on a 12-hr light–dark cycle with daily feedings of 6.5 mm Skretting "Sturgeon Broodstock" extruded slow-sinking pellets (Tooele, UT) at 1% of the tank's biomass. Pellets were fed by hand multiple times per day. Water was aerated via 30.5 cm-long low-pressure, high-volume porous air stones—dissolved oxygen was kept above 6 mg/L at all times. Each tank held four or five Canadian-origin Atlantic sturgeon with lengths ranging from 73 to 100 cm (ages estimated at roughly 3–5 years). Biomass varied across the two experimental mesocosms with 11.541 kg in tank 1 (ATT1) and 15.202 kg in tank 2 (ATT2). Prior to the start of the experiment, animals were fasted for 24 hr to reduce the amount of waste food accumulation in static tanks once the water was turned off.

On day 1 of the experiment (March 20), water was shut off for 26 hr to accumulate sturgeon eDNA (fish were not fed during this period). After 26 hr, fish were moved to new tanks with water flow and food restored. This period of time was assumed to achieve a "steady state" of eDNA concentration where production (shedding) was roughly balanced by degradation (decay) based on experiments by Sassoubre et al. (2016) that showed steady state was reached within ~16–36 hr for multiple fish species. Water in the tanks was then left for ~11 days to assess decay of eDNA after fish were removed. Water samples (1 L) were taken throughout the experiment starting on day 1 at 5.5, 11.5, and 26 hr (during eDNA accumulation) and then during the decay period at 32, 48, 75, 118.5, 191.5, and 268 hr (~11 days). Duplicate water samples in each tank (four total per time point) were collected from the surface in sterilized, 1-L wide-mouth polypropylene Nalgene bottles and filtered immediately or frozen until filtration. Water filtering, eDNA extractions, qPCR, and estimation of copy numbers were performed exactly as described above for qPCR of environmental samples. Due to the high concentrations of sturgeon eDNA in these mesocosms, most DNA extractions were diluted (up to 50-fold) with molecular grade water so that the input amount of total eDNA to any qPCR reaction was between 20 and 30 ng (estimated copy number and eDNA concentration were adjusted accordingly). Water samples were filtered on the same day or within one month of sampling (stored at −20°C), and filters were stored at −80°C until extraction and qPCR analysis, which was within days or weeks of water filtering.

2.2.8 | Calculation of shedding rates

Following the methods of Sassoubre et al. (2016), each tank was modeled as a mixed batch bioreactor with the equation:

$$V\frac{dC}{dt} = S - \mathrm{kCV} \quad (1)$$

where V is the volume of a given tank (in L), C is the concentration of eDNA in the tank (pg/ml), t is the time (hr) since the start of the experiment, S is the eDNA shedding rate (pg/ml/hr), and k is the first-order decay rate constant. At steady state, the change in eDNA concentration is zero ($\Delta C/\Delta t = 0$) and shedding rate is $S = \mathrm{kCV}$. The first-order decay rate constant was calculated for each tank separately, during the decay period (after fish were removed from the tank; hour 26–191.5) by linear regression of $\ln(C/C_o)$ versus time (hr) where C_o is the average eDNA concentration at 26 hr (assumed to be roughly steady state). Data across tanks were combined to assess differences in decay rates between tanks via ANCOVA (i.e., testing the significance of Tank * Time interaction). Finally, shedding rates were calculated for each tank and adjusted for biomass, with standard deviations determined by propagating errors for each multiplied term (k, C, and V; e.g., Sassoubre et al. 2016), which yields a relative standard deviation that is converted to ±standard deviation of the mean. All statistical analyses (linear regression, ANCOVAs) and plotting were carried out in R (R Core team, 2018).

3 | RESULTS AND DISCUSSION

3.1 | Initial qPCR assay testing

Initial tests of the CytB qPCR assay on Atlantic sturgeon DNA and DNA from a range of nontarget fish demonstrated that the assay is highly specific to Atlantic sturgeon, efficient, and has low

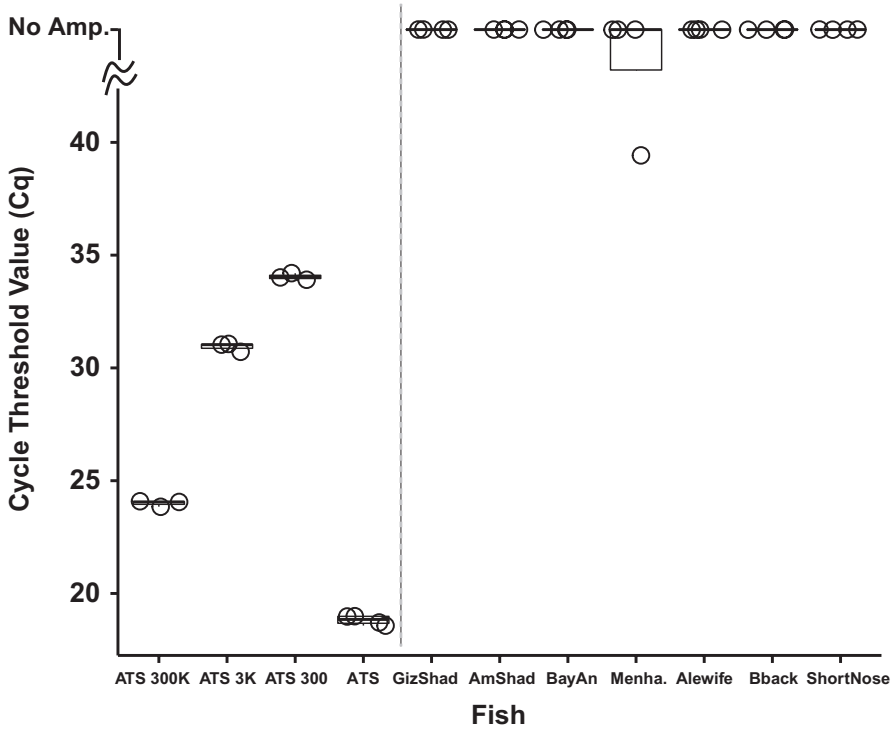

FIGURE 2 qPCR amplification results for the Atlantic sturgeon eDNA assay across a range of DNA samples. Cycle threshold values (Cq) for each reaction (run in triplicate for the oligo standards ATS 300K-ATS 300, quadruplicate for fish fin clip DNA) are plotted as open circles overlayed on a box and whisker plot (lower quartile box only visible for the menhaden sample). Samples to the left of the dotted gray line are amplification results (positive) for Atlantic sturgeon DNA: "ATS 300K," "ATS 3K," and "ATS 300" represent the 300,000 copy synthesized Atlantic sturgeon DNA oligo standard, the 3,000 copy oligo standard, and the 300 copy oligo standard, respectively. "ATS" is fin clip DNA from two different Atlantic sturgeon samples, each run in duplicate. Fish samples to the right of the gray line are results for nontarget fish; "GizShad" is gizzard shad (*Dorosoma cepedianum*), "AmShad" is American shad (*Alosa sapidissima*), "BayAn" is bay anchovy (*Anchoa mitchilli*), "Alewife" is alewife (*Alosa psuedoharengus*), "Bback" is blueback herring (*Alosa aestivalis*), and "shortNose" is shortnose sturgeon (*Acipenser brevisrostrum*). The Y-axis is broken above a Cq of 40 to allow plotting of nonamplifications (no detectable increase in fluorescence after 45 PCR cycles). For each sample, box and whisker plots are plotted with the points (bounding boxes represent the interquartile range values)

background amplification. As shown in Figure 2, there was no amplification of any of the nontarget fish (no amplification after 45 cycles) except for one qPCR replicate (of four) for Atlantic menhaden, which produced a Cq value of ~39. This was likely due to cross contamination as the sturgeon probe and primers combined have a total of 14 mismatches to menhaden Cytochrome B sequences. Shortnose Sturgeon, *A. brevirostrum*, did not amplify in any replicate across the eight samples tested. Atlantic sturgeon DNA amplified well (Cq values of ~18–22) for 10–20 ng DNA and showed a very strong concomitant linear decrease in Cq value with the log copy number for the synthesized oligo standard curve ($r^2 > 0.99$). Using Cq estimates for each sample from the standard curve (300–300,000 copies), calculated PCR efficiency of the assay was 97.97% with an amplification factor of 1.98.

3.2 | eDNA detection in environmental samples

Atlantic sturgeon eDNA was detected in 100% of water samples taken from the earthen pond, with a wide range of calculated starting copy numbers (eDNA abundance), from 980 copies/L to 25,341 copies/L (Table 2). eDNA abundance was much higher in the pond samples compared with the river samples (see below), which likely reflects the relatively high biomass/volume ratio in the ponds (~0.2 g/L). All seven detections were Sanger sequenced and searched against the NCBI "nt" database via BLAST, which confirmed that the positive qPCR detections in the pond were of Atlantic sturgeon DNA (see Appendix S1, Table A1).

Field sampling of water from two rivers in the Chesapeake Bay provided a more realistic and useful test for the qPCR assay. Samples taken during the summer (14 July 2016) on the Marshyhope Creek produced no positive qPCR detections (0/12; Table 2). The water blank (negative control) did not amplify. No PCR inhibition was detected for any of the environmental samples taken in the summer. The lack of eDNA detections largely corresponded to the lack of acoustic detections based on acoustic receiver data from 14 July 2016 at six sites in the Nanticoke River and Marshyhope Creek, with two exceptions (Table 2, Figure 3). Chapter Point had four sturgeon detected within the detection area, and Ferry Point had one sturgeon detected. The lack of eDNA detections is not surprising, since both locations are very deep (e.g., up to 17 m at Chapter Point; Appendix S1, Figure A1) within wide areas of the river, and

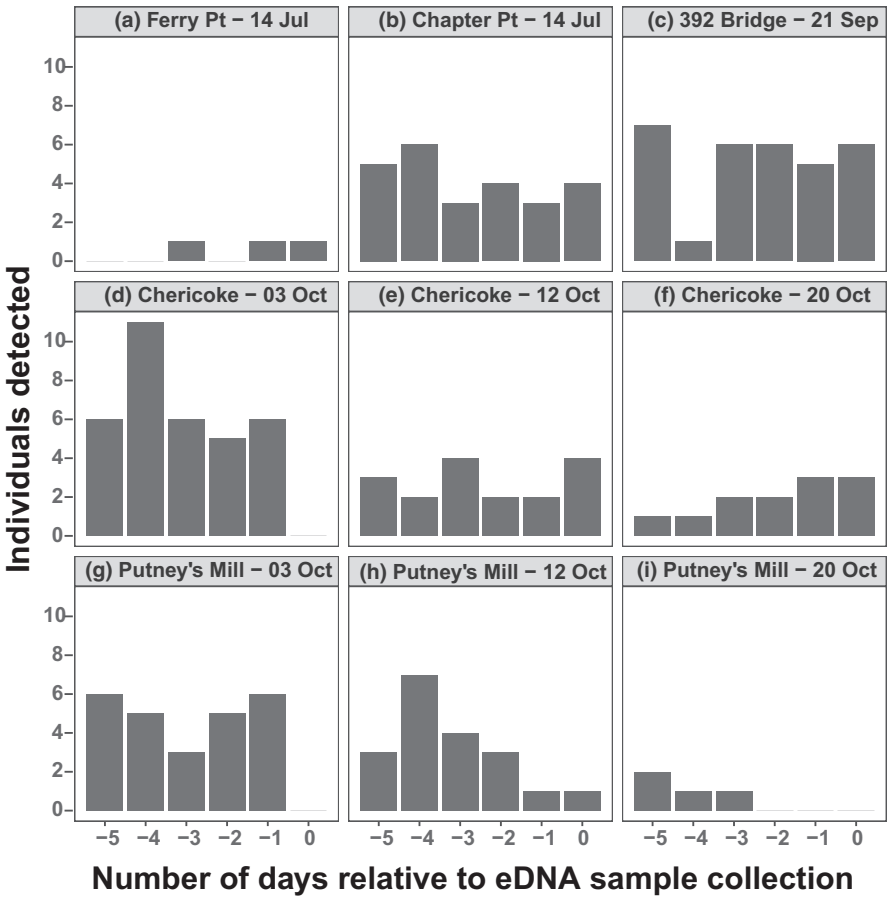

FIGURE 3 Daily number of individual (unique) Atlantic sturgeon detections from passive acoustic receivers in the Nanticoke River and Marshyhope Creek MD (Panel A-C) and the Pamunkey River VA (Panel D-I) over the previous five days and on the current day of eDNA sampling (day 0). Each panel represents a different site and date on which Atlantic sturgeon were detected from acoustic data. Sites with zero acoustic detections but receivers present (see Table 2 and Methods) were not included in the figure

eDNA samples were taken at the surface. During this time of the year, acoustic detection data suggest that sturgeon tended to rest in the deep channel of this river prior to continuing their up-migration to spawning grounds (Horne & Stence, 2016). It is also important to note that receivers only detect tagged sturgeon, so untagged sturgeon in the system would be missed by these methods, though activity patterns of tagged sturgeon are likely broadly reflective of all sturgeon, tagged and untagged, in the system.

In contrast, eDNA samples taken during the fall at the same sites on the Marshyhope Creek and on the Pamunkey River produced a number of positive eDNA detections. Two out of the five samples taken on 21 September 2016 on the Marshyhope Creek produced positive qPCR detections of Atlantic sturgeon (40%), both of which were taken at the Route 392 Bridge near Hurlock, MD (Figure 1, Table 2). Data from the passive acoustic receiver ~700 m downstream of the bridge confirmed significant sturgeon activity (at least seven fish) in the area on the day of eDNA sampling and in previous days, corroborating Atlantic sturgeon presence in this part of the creek (Figure 3). Reamplification of eDNA from these two positive detections produced readable sequence data that matched (via NCBI BLAST) to Atlantic sturgeon sequences in NCBI GenBank (percent identity >98%, e-value <1e−17; Appendix S1, Table A1). Again, no PCR inhibition was detected for any of the river samples taken in the fall.

Out of the 18 environmental water samples taken on the Pamunkey River in October, four had positive Atlantic sturgeon eDNA detections (~22% detection rate). Three of the detections occurred on October 3, and one on October 12. None of the three control bottles produced any amplification. Similar to detections from the Marshyhope Creek, eDNA abundances were much lower compared to the pond detections (Figure 5), likely reflecting the much lower biomass to volume ratios present in these river systems. No significant difference in eDNA abundance was detected between rivers (ANOVA $p > .05$). Acoustic data receivers proximal to water collection sites on the Pamunkey River showed that Atlantic sturgeon were active in the area prior to and on the day of eDNA collection (Figure 3), which provides corroborating evidence for the presence of Atlantic sturgeon in this system. Sanger sequences were produced for all four of the positive eDNA samples, and all of them matched Atlantic sturgeon sequences in NCBI at high percent identity (percent ID = 100%, e-value < 1e−37; Appendix S1, Table A1).

Quantitative comparisons of eDNA detection or abundances versus acoustic detections at all sites (both rivers) revealed significant relationships between eDNA and sturgeon presence (acoustic detections). First, simple linear regression of eDNA copy number versus cumulative acoustic detections of individual tagged sturgeon up to two or five days prior to the eDNA sampling showed significant, positive relationships ($p = .0071$ for two days, $p = .0023$ for five days; Figure 4a and b). Nonparametric correlations between the acoustic detections and eDNA abundance were significant and stronger for the five-day cumulative data (Spearman's Rho = 0.37, $p = .0336$ for two days; Spearman's Rho = 0.60, $p = .0002$ for five days). Logistic

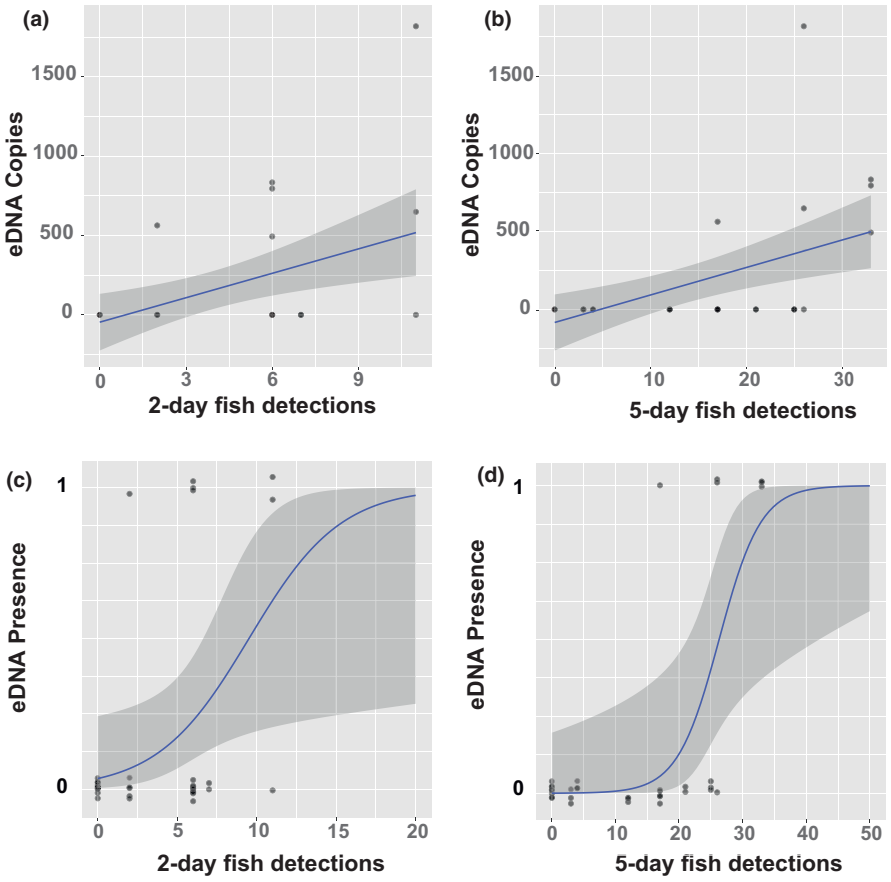

FIGURE 4 Linear and logistic regression analyses of acoustic versus eDNA detection data for Atlantic sturgeon. Panel A and B depict the least-squares linear regression lines (blue) and their standard error (shaded gray) for the cumulative number of daily, unique sturgeon detected via passive receiver up to two days or five days prior to taking eDNA samples, respectively. Panel C and D depict the GLM logistic regression results for acoustic detections (two-day or five-day cumulative unique detections, respectively) versus the presence (1) or absence (0) of a positive eDNA detection. Predicted probability of eDNA detection is plotted as the blue line, and standard error is represented by the shaded gray area. For Panels C and D, points are jittered vertically to reduce overplotting

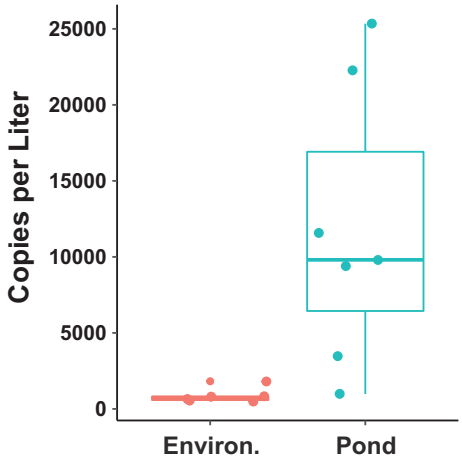

FIGURE 5 Comparison of eDNA abundances in pond detections versus environmental (river) detections. Bounding lines in box and whisker plots represent the 1st (lower) and 3rd (upper) quartiles, the thick lines in the middle of the box represent the median value, and "whiskers" extend to within 1.5 times the interquartile range of *Q1* and *Q3*. All values (copy number per 1 liter water filtered) are plotted over the box and whisker plots

regression was then used to assess the relationship between acoustic detections (two- or five-day cumulative detections of individuals) and sturgeon eDNA detection as a binary response variable (presence/absence; Figure 4c and d). Both two-day and five-day acoustic detections were significantly associated with the binary probability of eDNA detection (overall model chi-square p = .02 and .00007, respectively), producing sigmoidal relationships (greater binomial probability of eDNA detection with increased acoustic detections; Figure 4c and d). The two-day and five-day acoustic count variable had odds ratios (OR) of 1.40 and 1.36, respectively, indicating that a one unit increase in acoustic detections increases the odds of eDNA detection by 1.40 or 1.36, respectively.

Relatively few studies have employed acoustic detection and eDNA analysis at the same time, or directly compared species detections from each data type. Foote et al. (2012) compared acoustic and eDNA detections of harbor porpoises in net pens and in open sites in the Baltic Sea, finding that acoustic data were much better at detecting porpoises in the open Baltic sea sites and that eDNA often failed to detect individuals when they were present. Takahara et al. (2020) also found relatively weak relationships between acoustic detections of three endangered frogs (male mating calls) and eDNA. However, potential biases in the type of acoustic data collected (adult male mating calls) may have led to a lack of association with eDNA, which would represent occupancy of frogs from any life-stage and either sex, not just adult males for the acoustic detections. The acoustic dataset used in the present study also has some limitations as receivers only detected previously tagged Atlantic sturgeon, so any untagged sturgeon in the system would have been missed (not recorded). Nevertheless, the presence of multiple passive acoustic arrays proximal to our eDNA sampling sites allowed us to utilize highly spatially resolved acoustic data for Atlantic sturgeon that appeared to correlate well with sturgeon eDNA presence and relative abundance.

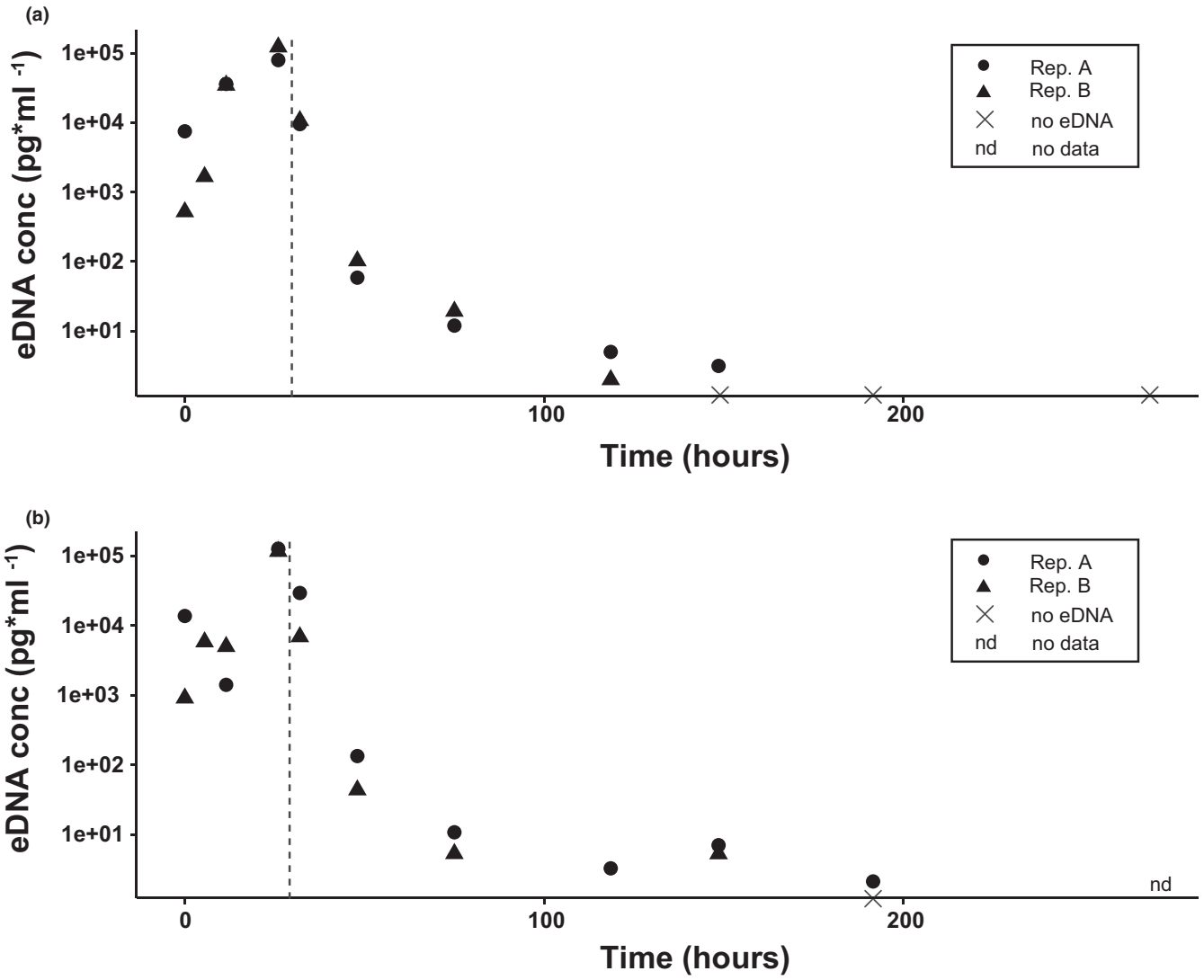

FIGURE 6 Atlantic sturgeon eDNA concentrations in shedding rate mesocosm experiments over time. Each point is the estimated eDNA concentration (pg/ml) from one of the duplicate 1-L water samples (replicate A: filled circles, replicate B: filled triangles) over time, for each tank. Each Panel represents results for the two separate tanks (Panel A, ATT1; Panel B, ATT2). The dashed line at 26 hr denotes the point in the experiment where fish were removed from the mesocosms. "×" symbols denote time points (or replicates) that had no detectable Atlantic sturgeon eDNA and "nd" denotes time points that lacked data. The Y-axis is plotted on a log scale

Overall, given the relatively limited field sampling efforts in each river, the initial eDNA results for these systems are encouraging and suggest that Atlantic sturgeon can be detected in rivers with eDNA analysis. Moreover, given that eDNA abundances were moderately (positively) associated with the abundance of tagged sturgeon near the sites of water sampling, eDNA likely has some predictive power to estimate occupancy. Based on these preliminary analyses, eDNA detection appears to be more likely when Atlantic sturgeon are in greater abundance, actively migrating or spawning, and perhaps in lower river volume. When sturgeon were in lower abundance and residing in deeper and larger parts of the river (e.g., July 14 samples on the Marshyhope Creek), eDNA sampling was less likely to detect the fish when they were actually present. Greater spatiotemporal representation (e.g., eDNA sampling at multiple depths and more frequently) may improve detection accuracy for these systems. Sampling sediment for sturgeon eDNA could also increase the likelihood of detection as eDNA can be 10- to 1000-fold more concentrated than aquatic samples and persist for months (e.g., Turner et al. 2015). However, sedimentary eDNA detections would not necessarily reflect recent occupancy and thus would be better suited to guide future sampling of the water column at sites where sediment eDNA was detected.

Finally, it is important to briefly discuss the potential impact of physical and environmental factors on Atlantic sturgeon eDNA detections, which were not explicitly examined in this study. First, the study did not utilize information on the potential for transport of Atlantic sturgeon eDNA downstream of sites they occupy and how flow rate and hydrology of the rivers might affect eDNA detection probability (i.e., what is the residence time, transport, and resuspension of Atlantic sturgeon eDNA in these systems; e.g., Shogren

TABLE 3 eDNA decay and shedding rate estimates for Atlantic sturgeon

Tank	k[a]	*SD k*	Shedding rate (pg/hr)	±*SD*[b]	KG fish	Shedding rate (pg/hr/g fish)	±*SD*[c]
ATT1	−0.084	0.017	2.39E + 07	8.94E + 06	11.541	2067.6	774.37
ATT2	−0.06	0.014	2.02E + 07	5.48E + 06	15.203	1,331.106	360.213

[a]Decay rate constant estimated for each tank.; [b]Standard deviation (*SD*) of the gross tank shedding rate (pg/ml/hr).; [c]The relative standard deviation of the biomass-adjusted shedding rate (pg DNA/hr per gram biomass of fish) calculated based on propagation of the error for each parameter.

et al. 2017). Estimating transport and residence time of sturgeon eDNA in these river systems is certainly an important area of future work and will require more highly resolved spatial and temporal sampling and estimates of flow rate. It is also important to acknowledge that water quality parameters in these rivers, such as temperature, pH, and dissolved oxygen concentration, could also have impacted the persistence or generation of Atlantic sturgeon eDNA and thus eDNA detection probabilities. With expanded, future eDNA monitoring for Atlantic sturgeon in these rivers, water quality parameters should also be measured to examine their potential covariation with patterns of eDNA abundance and persistence.

3.3 | Shedding and decay rate of Atlantic sturgeon eDNA

Atlantic sturgeon eDNA concentration increased over the first 26 hr after water was turned off and came toward an apparent steady state in tanks ATT1 and ATT2, with average concentrations of 101,699 and 120,487 pg/ml of eDNA, respectively. As eDNA accumulated in these early time points, a hazy film developed on the water surface which may have been from mucous released by the fish. Given that sturgeon lack scales (a potential source of eDNA in other fishes when shed into the environment; e.g., Rees et al. 2014; Sassoubre et al. 2016), eDNA likely came from mucous secretion and urine excretion—very little particulate waste was observed in tanks. After removing fish, eDNA declined steadily over the next eight days (Figure 6), becoming undetectable by day eight (hour 191.5) in ATT1 and near zero (no detection and 912 copies in the two replicates, respectively) in tank ATT2. First-order decay rates were calculated for each tank during this period (26–191.5 hr) and were highly significant, with moderate to strong linear fits (p = .00066, R^2 = 0.71 for ATT1, p = .002, R^2 = 0.59, for ATT2). Decay rate constants (k) were fairly consistent among tanks, ranging from 0.059 to 0.084 (Table 3). Analysis of covariance on the combined dataset revealed no significant difference in decay rates among tanks (Tank * Hour term p = .31). eDNA shedding rates were similar across the two tanks and were estimated at 2067.6 pg DNA/hr per gram biomass (±774.37) for ATT1 and 1,331.11 pg DNA/hr per gram biomass (±360.21) for ATT2 (Table 3).

The eDNA shedding rates estimated for Atlantic sturgeon in this experiment were slightly higher but on the same order of magnitude as those reported for other marine fishes in Sassoubre et al. (2016), which ranged from 165 pg hr^{-1} g^{-1} (northern anchovy, *Engraulis mordax*) to 3,368 pg hr^{-1} g^{-1} for the Pacific sardine (*Sardinops sagax*). Importantly, we used a very similar design and almost identical analyses of shedding and decay rates as Sassoubre et al. (2016); thus, the shedding rates estimated for Atlantic sturgeon can be directly compared to these other rates. Based on our experimental results, Atlantic sturgeon produced eDNA at a similar, or in some cases greater, rate to these other fishes, all of which have scales. Although the shedding of scales has been considered a potential source of eDNA for fishes (e.g., Rees et al. 2014), studies of catfish (which lack scales) and eels (which have embedded scales less likely to be shed) have shown that eDNA can still be reliably detected from these species in aquatic environmental samples (e.g., Eva et al. 2016; Kasai et al. 2020; Takeuchi et al. 2019). Thus, detection of Atlantic sturgeon via eDNA analysis of environmental water samples should be as effective as for any other fish of interest. Relatively weak detections of Atlantic sturgeon eDNA in samples from the two rivers are not likely to be a function of reduced eDNA shedding by Atlantic sturgeon but instead could be due to a relatively low abundance of sturgeon and (or) the hydrography of the rivers we sampled. One important caveat to our shedding rate estimates is that steady state (i.e., decay = shedding) may not have been achieved during the accumulation phase of the experiment (while fish were in tanks with the water turned off). If eDNA concentration was still increasing, then the average concentration, C, at steady state may have well been higher, which would increase the overall estimate of shedding rate (shedding rate, S = kCV, in Equation 1). Thus, these estimates of shedding rate may represent a lower bound for Atlantic sturgeon.

First-order decay rates in our experiment (k ~ 0.059–0.84) were also similar to estimates in Sassoubre et al. (2016; 0.055–0.101) indicating potentially similar environments for eDNA decay despite differences in water chemistry and salinity (salinity of 5–7 here versus 31–39 in Sassoubre et al. 2016). eDNA decay is expected to be higher in marine environments compared with freshwater river or lake environments (e.g., Dell'Anno & Corinaldesi, 2004; Thomsen, Kielgast, Iversen, Moller, et al., 2012), though a recent literature review in Collins et al. (2018) suggests the opposite might be true, with acidic streams having the fastest degradation rates reported (e.g., half-life of <1.2 hr; Seymour et al. 2018). Our study used local (natural) water pumped directly from the Choptank River, a major tributary of the Chesapeake Bay estuary that would be considered "mesohaline" in the midbay region where the Choptank River is located (salinity range of 5–18; salinities ranged from 5 to 7 during the experiment). Few eDNA decay studies have been conducted in estuarine waters with salinities between 3 and 20 (Collins et al. 2018). Thus, data on eDNA decay rates for these environments are lacking. The similarity of decay rates from our experiment at relatively low

salinity and Sassoubre et al. (2016) at high salinity (coastal oceanic water, >30) may also be driven by the fact that our studies used similar sized tanks and volumes (i.e., similar surface area to volume ratio), which could have impacted bacterial activity and mixing rates. Compared with persistence times of ~8 days for Atlantic sturgeon eDNA measured here, eDNA persistence was shown to be slightly higher for Siberian sturgeon (*Acipenser baerii)* in freshwater ponds, in which sturgeon eDNA was present after 14 days (Dejean et al. 2011). This difference in persistence could be due to a number of different factors, including different shedding rates between the species, but it may also reflect greater persistence of sturgeon eDNA in freshwater ponds.

4 | CONCLUSIONS

In this study, we developed a robust qPCR assay for detecting Atlantic sturgeon eDNA in field samples that was highly efficient and species-specific. Trials of the qPCR assay on water samples taken from an earthen pond with a high density of sturgeon produced relatively strong detections in all samples (1,000s of copies per liter), while qPCR of environmental collections from two tidal rivers produced weaker detections (100s of copies per liter) in 26% of samples taken during fall spawning runs. Quantitative comparisons of eDNA detection or abundances versus acoustic detections at all sites (both rivers) revealed significant relationships between eDNA and sturgeon presence based on acoustic data. However, when sturgeon were at lower abundances, not actively migrating, and in deeper portions of the river, eDNA analysis from surface water samples failed to detect Atlantic sturgeon for some samples. Finally, estimates of Atlantic sturgeon eDNA shedding rates were comparable to those of other marine fishes, suggesting that low abundances, seasonal differences in behavior, and hydrography of rivers may limit sturgeon eDNA detections in some cases.

Overall, these results indicate that qPCR-based eDNA analysis of Atlantic sturgeon has broad potential application. eDNA is a viable approach for monitoring this important endangered species and can inform DNA-based trophic dynamics studies (Bunch et al. 2021). Given the relatively low eDNA abundances in river samples, increasing the water volume sampled, the sampling frequency, and varying the depths sampled may improve detection probability. Applying these methods throughout Chesapeake Bay tributaries and in other DPS along the Atlantic Coast is an important next step in documenting Atlantic sturgeon distribution, which will inform management decisions (e.g., time-of-year restrictions), and identify areas of interest for further population dynamics studies. More broadly, the successful application of eDNA analysis for an endangered fish species at low abundances indicates that this method could be applicable to other fish species with similar threatened or endangered status.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

LVP designed the study, assisted with data analysis, field, and laboratory work, drafted the initial version of the manuscript, and contributed to revisions; AB contributed to data analysis, environmental sampling, initial drafting of the manuscript, and revisions to the manuscript; BL and CF performed laboratory work and data analysis and contributed to writing/revision of the manuscript; CS and BR contributed to environmental sampling, data analysis, and revisions to the manuscript.

DATA AVAILABILITY STATEMENT

Data associated with this study are archived at datadryad.org: (https://doi.org/10.5061/dryad.q83bk3jgs).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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