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# Saving the doomed: Using eDNA to aid in detection of rare sturgeon for conservation (Acipenseridae)

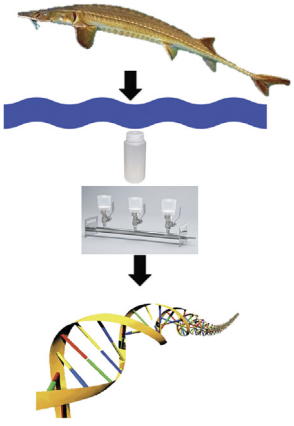
Mariah O. Pfleger [a], Steven J. Rider [b], Carol E. Johnston [c], Alexis M. Janosik [a,*]

[a] Department of Biology, University of West Florida, Pensacola, FL 32504, USA
[b] Alabama Division of Wildlife and Freshwater Fisheries, River and Stream Fisheries Lab, 3608 Fairground Road, Montgomery, AL 36110, USA
[c] Fish Biodiversity Lab, Department of Fisheries, Auburn University, Auburn, AL 36849, USA

## HIGHLIGHTS

- Successful detection of Alabama and Gulf sturgeon using eDNA.
- Distribution and temporal data suggest individuals of both species may have migrated through or over navigation locks or dams and remained upstream of passage barriers.
- The distribution of these species suggests that the removal of at least one passage barrier will benefit both of these imperiled fishes.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Environmental DNA (eDNA) is a relatively new technique that has proven to be a successful tool for the detection of rare and/or spatially and temporally variable organisms. For aquatic species, field sampling can require extensive effort and may be unreliable in terms of determining the presence or absence of a target species, especially when the target species is rare. For this study we used eDNA to try to detect Alabama Sturgeon (*Scaphirhynchus suttkusi*) and Gulf Sturgeon (*Acipenser oxyrinchus desotoi*) presence in the Mobile River Basin of Alabama. These two sturgeon species make ideal model organisms for examination of this technique in the detection of rare species, as the Alabama Sturgeon is critically endangered and the Gulf Sturgeon is listed as vulnerable on the International Union for Conservation of Nature (IUCN) Red List. In spite of the critical need for information on these species, riverine sampling is expensive and produces low detection. Results using

* Corresponding author
*E-mail address:* ajanosik@uwf.edu (A.M. Janosik).

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eDNA have revealed temporally logical, positive detections of Alabama and Gulf sturgeon throughout the Mobile River Basin sites included in this study. Successful detection of these species could reveal vital information such as understanding of habitat use for management purposes as well as identify specific localities for field sampling. Removal of at least one passage barrier will benefit both of these imperiled fishes.


## 1. Introduction

With over 85% of species listed as endangered or threatened, sturgeon are considered the world's most imperiled vertebrate group (IUCN Red List). All species of sturgeon are migratory, as either potamodromous or as anadromous fishes. As a result, sturgeon species are negatively affected by passage barriers, such as navigation locks and dams (Boreman, 1997). Furthermore, reservoirs created as a result of damming are unsuitable habitat, especially for larvae that drift downstream from suitable, free-flowing river reaches. As long-lived fishes that do not breed annually, sturgeon populations are slow to recover from years of poor recruitment. Moreover, many species of sturgeon are harvested for caviar, and their lack of resiliency from harvest, in conjunction with these other factors, has led to many species being on the brink of extinction.

Suspected near extinction for many years, the Alabama Sturgeon (*Scaphirhynchus suttkusi*) is the rarest and most endangered species of this imperiled group of fishes (Burke and Ramsey, 1985; Parauka, 2004; Rider and Hartfield, 2007; Kuhajda et al., 2009). The Alabama Sturgeon is potamodromous, migrating within the river system to breed and forage and currently found only in the Mobile River Basin of Alabama (USFWS, 2008). Only seven specimens have been collected since 1997, five by Alabama Division of Wildlife and Freshwater Fisheries (DWFF) biologists and 2 by commercial fishers (Rider and Hartfield, 2007; Rider et al., 2011). The last specimen was collected from the Alabama River on April 3, 2007 (Rider et al., 2011). Another specimen was observed below Robert F. Henry Lock and Dam on April 23, 2009; however, DWFF biologists were unable to net the fish (Rider et al., 2010). Thus, in spite of extremely intensive efforts to collect this species for broodstock and habitat information, the species is currently undetectable using conventional sampling. Although it is suspected that Alabama Sturgeon are negatively affected by numerous passage barriers preventing upstream migration to spawn in the Alabama River system, the species' range, the inability to collect the species makes conservation decisions challenging. Information on movement patterns is critical to management recommendations such as dam removal. It is possible, given their long life spans, that some sturgeon were also trapped upstream of dams and are unable to move downstream.

The Gulf Sturgeon (*Acipenser oxyrinchus desotoi*) is an anadromous species that occurs along the northern Gulf of Mexico. It is federally listed as threatened and state protected in Alabama. This species spawns in freshwater rivers in the spring and remains through the summer; then migrates to marine and estuarine habitats in the fall and winter to feed (Huff, 1975; Wooley and Crateau, 1985; Fox et al., 2002). The occurrence of Gulf Sturgeon in the Mobile River Basin has been considered rare (Boschung and Mayden, 2004). Mettee et al. (2011) sampled for Gulf Sturgeon from 2005 to 2008 and caught only 2 specimens; albeit, both were collected from Mobile Bay. Although recent sampling efforts have not collected any Gulf Sturgeon, an acoustic array in the basin has detected sonic-tagged Gulf sturgeon from other river systems (Rider et al., 2013, 2014, 2015, 2016). These results indicate the Mobile River Basin may in fact be an important summer habitat or offer spawning habitat for Gulf Sturgeon.

An alternative approach for monitoring of rare or elusive species is through the use of environmental DNA (eDNA), i.e. the extraction and analysis of genetic material obtained directly from environmental samples from sloughing of skin cells, intestinal cells, scales, and/or mucus. For macro-fauna, this approach was first applied to terrestrial sediment samples revealing the presence of mammals, birds, and plants (Willerslev et al., 2003). More presently, the same approach has been used to detect invasive and imperiled freshwater fishes (Ficetola et al., 2008; Jerde et al., 2011; Takahara et al., 2012; Thomsen et al., 2012b; Janosik and Johnston, 2015; Sigsgaard et al., 2015; Boothroyd et al., 2016). Water samples, rather than direct species contact, can be used to identify which species have recently been present in a local environment. Sampling free eDNA in water is potentially faster, less expensive, and less destructive than traditional sampling methods. Further, eDNA allows for species detection without the need to capture individual specimens, avoiding handling stress and/ or mortality, particularly when the species is rare or elusive (Thomsen et al., 2012a). In order to provide distributional information critical to management of both of these rare sturgeons, our objective was to use environmental DNA (eDNA) as a detection tool for these species in the Mobile River Basin (i.e., Alabama, Tombigbee and Cahaba rivers). We sampled during the winter (non breeding season) of 2014 and spring (spawning season) of 2015 with an aim to better understand the timing of spawning migrations, temporal and spatial distribution of these species.

## 2. Methods

A total of 130 water samples for eDNA analysis were collected from sites in the Mobile River Basin (Appendix A). Locations were selected based on known and historically documented occurrences of each species. Sampling sites are as follows (Fig. 1): Tombigbee River: Coffeeville Lock and Dam, River Mile 116, River Mile 110, River Mile 104, River Mile 98, River Mile 92, River

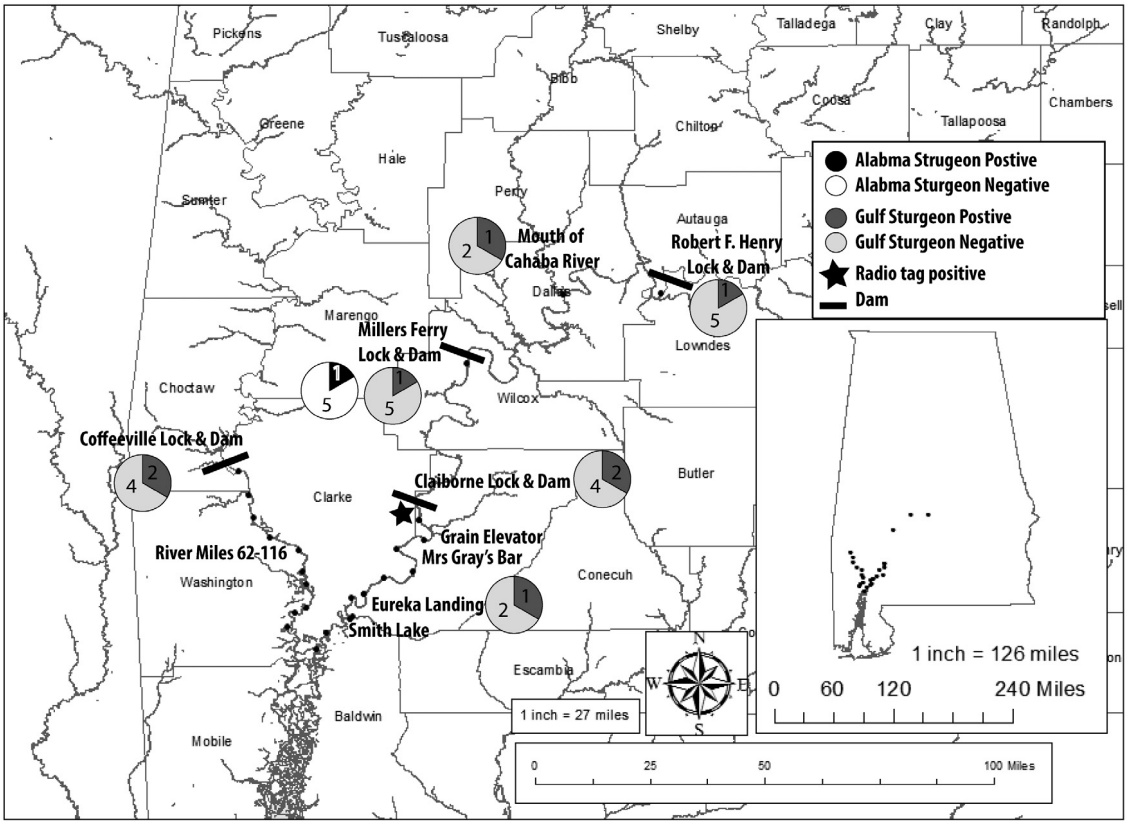


**Fig. 1.** Map of all positive sample locations of Gulf Sturgeon and Alabama Sturgeon using eDNA from December 2014. Small black circles represent localities sampled. Pie charts indicate both positive and negative samples taken at specific locations. Black indicates positives for Alabama Sturgeon, while white indicates negatives for Alabama Sturgeon. Dark gray indicates positives for Gulf Sturgeon, while light tray indicates negatives for Gulf Sturgeon. Lines indicate dams. The star indicates positive radio tag location. Rivers flow north to south.

Mile 622; Mouth of Cahaba River (Cahaba River), and the following sites on the Alabama River: Eureka Landing, Claiborne Lock and Dam, Millers Ferry Lock and Dam, Robert F. Henry Lock and Dam, Smith Lake, Grain Elevator, Mrs. Gray's Bar, Marshall Bluff, Upstream of Gaines Town, Across Sizemore Creek, Choctaw Bluff, Dixie Landing, Joe David Landing, ARL Cutoff to Tombigbee. In particular, two sites were sampled below Coffeeville (Tombigbee River rkm 256), Claiborne, Millers Ferry, and Robert F. Henry Locks and Dams (Alabama River rkm 134, 246 and 437), as dams can be potential barriers to dispersal. Sampling sites were located between 0.1 and 0.8 km below each lock and dam. Also, two sites were located at the mouth of the Cahaba River where it flows into the Alabama River (rkm 350) and near Eureka Landing on the Alabama River (rkm 74). Sampling sites in the Cahaba River and from each set of lock and dam were chosen to coincide with spawning migrations. We chose Eureka Landing for the last sampling site since this was the location where the last acoustically-tagged Alabama Sturgeon resided a majority of the time (S. Rider, DWFF, unpublished data).

Environmental DNA collection methods herein are similar to the procedure developed by Mahon et al. (2010) and Jerde et al. (2011). Two 1-liter water samples were collected from each site. At each major site, three sites replicates were sampled (ex 1A, 1B, 1C). Water sampling was carried out from December 11, 2014, and from April 14, 2015–July 16, 2015. Quality control measures, such as sterile technique for collecting and decontamination, per Mahon et al. (2010), were taken at each site to avoid contamination and reduce the possibility of false positives. A cooler storage control of $ddH_2O$ was employed for each sampling trip to ensure samples collected were not contaminated. Collected samples were immediately placed on ice in a sterilized ~50 L cooler storage container to prevent DNA degradation. Within 24 h of collection, water samples were vacuum-filtered onto 1.5 μm pore size glass fiber filters (Whatman™) using a filter funnel attached to a vacuum source by the Johnston Fish Biodiversity Lab (Auburn, AL). To ensure equipment control, deionized water was passed through each sterilized filter apparatus prior to each sample filtration and treated as a normal sample from here on out. After filtration, sample filters were placed in 50 ml conical tubes and stored at −20 °C until DNA extraction.

DNA was extracted from filters using the PowerWater DNA Isolation kit (MOBio Laboratories Inc., Carlsbad, CA) following manufacture's recommendations, with the exception of agitating samples for 30 min rather than 5 min, and the final DNA elution step. Final DNA elution was done using deionized water instead of the provided buffer solution. DNA was also extracted from reference samples of Alabama sturgeon, Gulf sturgeon, and using the DNeasy Blood and Tissue Kit (Qiagen®).

**Table 1**
Primer sequences Alabama Sturgeon (ALSturg), Gulf Sturgeon (GulfSturg) and Paddlefish.

| Primer | Sequence |
|---|---|
| ALSturgF | 5′-CTCCTAGGCCTCTGCCTTATT-3′ |
| ALSturgR | 5′CTTTTTGGAGGTAGGAGCCGT-3′ |
| GulfSturgF | 5′CGAGCGCTAGCTTAAAACCCA-3′ |
| GulfSturgR | 5′CCCATAGCCGGCTTCAAAAGA-3′ |
| PaddlefishF | 5′CGAGCGCTAGCTTAAAACCC-3′ |
| PaddlefishR | 5′CCCCATAGCCGGCTTCAAAA-3′ |

Extraction of DNA was confirmed by quantification using a Qubit 3.0 Fluorometer (ThermoFisher Scientific). Extraction blanks and cooler/filter controls were included for all DNA extractions and tested negative in subsequent PCRs.

To ensure species specificity we targeted a short fragment (~150 bp) of the cytochrome *b* gene of mitochondrial DNA and used the BLAST (Basic Local Alignment Search Tool; Genbank, www.ncbi.nlm.nih.gov). Molecular markers were designed with high specificity for Gulf Sturgeon and Alabama Sturgeon using Primer 3 (Rozen and Skaletsky, 2000) and Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), and were tested for cross amplification between the two target species. Paddlefish (*Polyodon spathula*), a common species in the same order as sturgeon, were included as a methods control as Paddlefish are abundant throughout the Alabama River system (Rider et al., 2012) and detected at several sites. Additionally, the markers were tested for cross amplification in Paddlefish and Lake Sturgeon (*Acipenser fulvescens*) to ensure marker specificity. No other sturgeon species are present in the Tombigbee, Alabama, or Cahaba Rivers. All positive bands were sequenced to further ensure marker specificity.

Presence of Gulf Sturgeon, Alabama Sturgeon, and Paddlefish DNA was assessed using polymerase chain reaction for each filtration control, water sample, and cooler control. Three PCR replicates were performed per water sample. Twenty-five microliter reactions were performed using 11 μl of deionized water, 1 μl of each primer (10 μM) (Table 1), and 10 μl of TaqMan® Environmental Master Mix 2.0 (Life Technologies). PCR amplification for both species took place under the following conditions: initial incubation at 95 °C for 3 min, followed by 32 cycles of 95 °C for 1 min, 58 °C for 1.5 min, and 72 °C for 3 min. Multiple PCR reactions were run for each sample to ensure validity of results (Ficetola et al., 2015). For each positive sample, three PCR reactions were performed. PCR results were visualized under UV light on a 1% agarose gel stained with ethidium bromide. Negative and positive controls were run for each gel. For all positive samples, not all replicates were positive, likely due to low concentration of DNA, but all positive samples were purified for sequencing. For all positive samples, PCR products were purified using Exonuclease I and Fast Alkaline Phosphatase (ExoFAP, ThermoFisher Scientific) or extracted from the agarose gel using the Qiagen QIAquick gel extraction kit (Qiagen, Inc., Valencia, CA) and bi-directionally sequenced by GenWiz, Inc. (New Brunswick, NJ). Sequences were proofread using Sequencher 5.1 (Gene Codes, Inc.) and aligned. For species identify verification, sequences were compared with GenBank Nucleotide database using BLASTn (Altschul et al., 1990) (see Appendix B). No evidence of contamination with sturgeon DNA was present at any step of sample collection, DNA extraction, or PCR.

## 3. Results

In December of 2014 (prior to the expected upstream migration of both species), 8 of 30 (27%) water samples collected were positive for Gulf Sturgeon DNA, while 1 of the 30 (3%) total water samples collected was positive for Alabama Sturgeon DNA. This positive for the Alabama sturgeon was from Miller's Ferry Lock and Dam site (32.08873°N, 87.40011°W), in the Alabama River. Specific numbers of positives and negatives by site are given in Fig. 1. Gulf Sturgeon detections in December were from the Alabama, Cahaba and Tombigbee Rivers at the following sites: Robert F. Henry Lock and Dam, Mouth of the Cahaba River, Millers Ferry Lock and Dam, Claiborne Lock and Dam, Eureka Landing, and Coffeeville Lock and Dam (Figs. 1 and 2).

From April to July of 2015 (when upstream migration is expected for both species), 43 of 100 (43%) water samples were positive for Gulf Sturgeon DNA and 17 of 100 (17%) were positive for Alabama sturgeon DNA. Specific numbers of positives and negatives by site are given in Figs. 3 and 6. For Gulf sturgeon, in the Alabama River, 22 of 61 (36%) water samples were positive, in the Cahaba River, two of three (67%) water samples were positive, and in the Tombigbee River, 19 of the 36 (53%) water samples were positive for Gulf sturgeon DNA (Fig. 4). Temporally, April samples yielded 20 positives (65%) from the Alabama, Cahaba, and Tombigbee Rivers. In May, 18 (30%) of samples were positive for Gulf Sturgeon DNA, but positives were only detected in the Alabama and Tombigbee Rivers. Lastly, in the month of July, five of nine (55%) water samples were positive for Gulf sturgeon DNA, but only in the Alabama River (Fig. 4). No positives were detected in July in the Cahaba and Tombigbee Rivers because collections were made only in Smith Lake in the Alabama River for July. Gulf Sturgeon DNA positives can be seen by site in Fig. 5. For Alabama sturgeon, in April, seven of the 31 (23%) samples collected were positive, six of the 60 (10%) samples collected in May were positive, and four of 9 (44%) samples collected in July were positive for Alabama Sturgeon DNA (Fig. 7). Positives were detected in the Alabama River at the following sites: Robert F. Henry Lock and Dam, Millers Ferry Lock and Dam, Claiborne Lock and Dam, Choctaw Bluff, Across Sizemore Creek, Eureka Landing, and Smith Lake (Fig. 7).

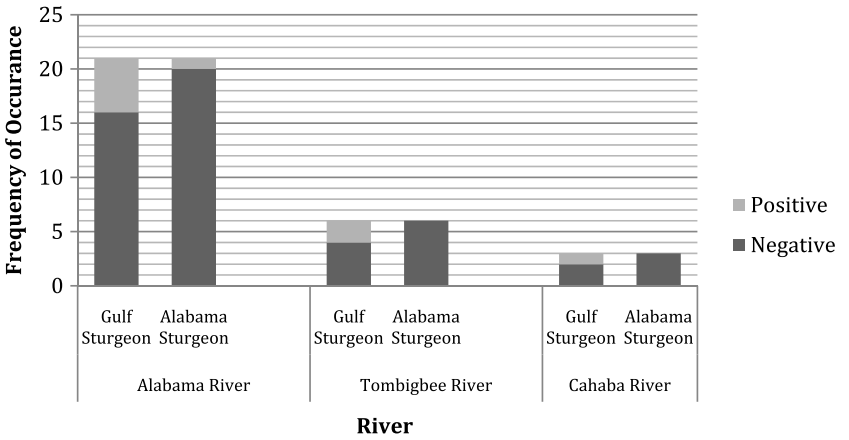


**Fig. 2.** Number of Gulf and Alabama Sturgeon positives in December 2014 by river. Light gray indicates positive samples, while dark gray indicates negative samples.

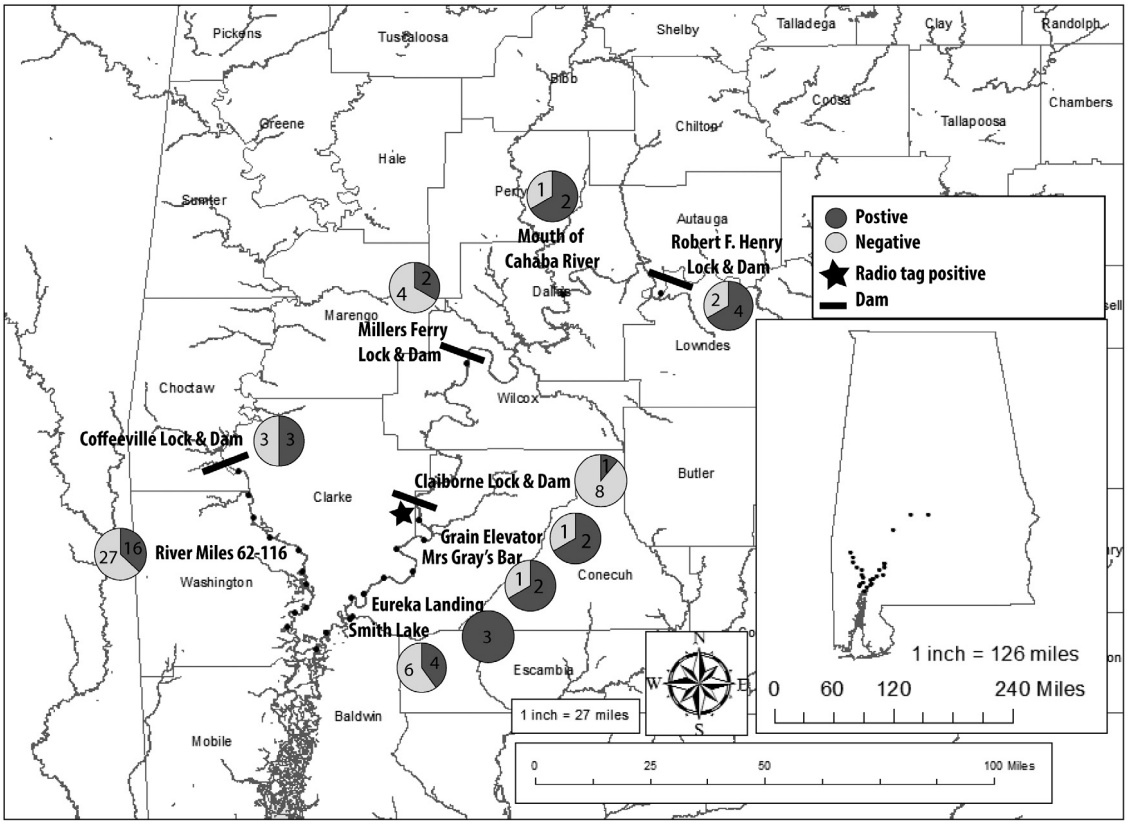


**Fig. 3.** Map of all positive sample locations of Gulf Sturgeon using eDNA from April, May and July of 2015. Black circles represent localities sampled. Pie charts indicate both positive and negative samples taken at specific locations. Dark gray indicates positives, while light tray indicates negatives. Lines indicate dams. The star indicates positive radio tag location. Rivers flow north to south.

## 4. Discussion

This study demonstrated, through the use of eDNA, that Alabama Sturgeon are still extant in the Alabama River system. Alabama Sturgeon are exceptionally rare and therefore virtually impossible to detect using traditional sampling methods. Between 1997 and 2005 over a period of 2447 man-days, a number of agencies collected only five Alabama Sturgeon using traditional field sampling (Rider and Hartfield, 2007). However, using eDNA, several detections of Alabama Sturgeon were recovered. Environmental DNA detection of Alabama Sturgeon during the non-breeding season between two passage

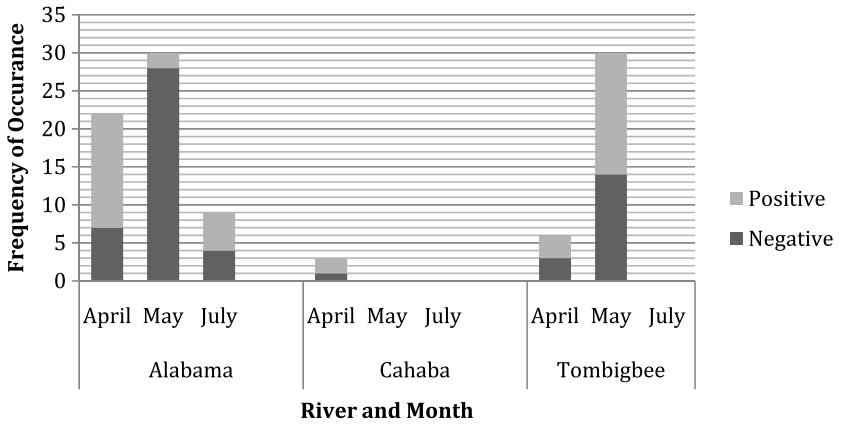


**Fig. 4.** Number of Gulf Sturgeon positive detections by river and month. Light gray indicates positive samples, while dark gray indicates negative samples.

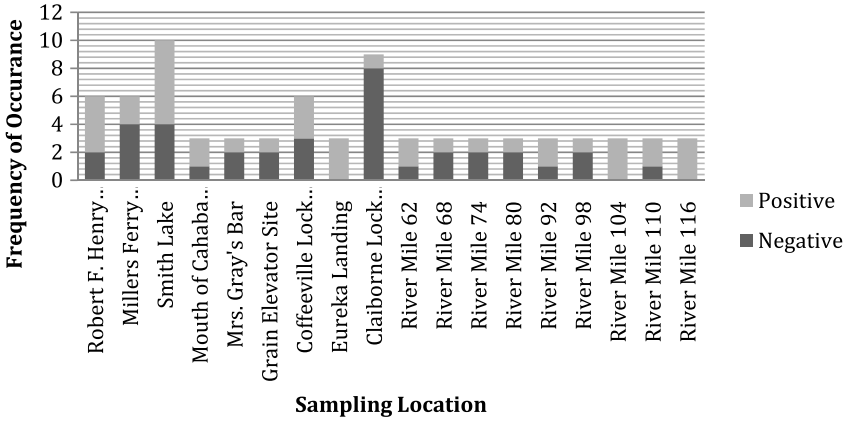


**Fig. 5.** Number of Gulf Sturgeon positive detections by site. Light gray indicates positive samples, while dark gray indicates negative samples.

barriers suggests that individuals may be trapped in a reservoir. Alternatively, the species may be traversing the spillway or navigation lock and dam at Claiborne. These possibilities are true during the spring spawning season as well. Although most eDNA detections were from areas below the first passage barrier on the Alabama River (Claiborne lock and dam), there were eDNA detections past two passage barriers. Temporally, more eDNA detections were from the spring spawning season.

Similar spatial and temporal patterns were observed for Gulf Sturgeon. Individuals were detected above passage barriers in both winter and spring samples, with most detections occurring below the first passage barrier in spring. Historically, Gulf sturgeon are known enter rivers from the Gulf of Mexico to spawn between the months of February and April and remain in rivers until October and November when they return to the Gulf to feed on amphipods, isopod, midges, crabs and shrimp (Foster and Clugston, 1997; Sulak and Clugston, 1999; Fox et al., 2000; Ross et al., 2009). Gulf sturgeon show migratory movement over a temporal scale. Specifically, in the Alabama River, the majority of positive samples were detected in April compared to May and July, while in the Tombigbee River, the majority of positive samples were detected in May rather than April. In addition, Gulf Sturgeon at Claiborne Lock and Dam were detected both by eDNA and by sonic tag (Rider et al., 2016), further corroborating detection by environmental DNA.

Since 2010, the US Army Corps of Engineers in cooperation with the Alabama Department of Conservation and Natural Resources has been conducting voluntary conservation locking measures to provide potential fish passage during the spring spawning season at Clairborne and Millers Ferry lock and dam. The detection of Alabama and Gulf sturgeon eDNA above these hydro projects could indicate the potential for sturgeon to pass through these navigation locks. However, further study is needed to determine the correct path of passage and to what extent.

The goals of conservation efforts for Alabama sturgeon and Gulf sturgeon include prioritization of habitat protection, and establishing a captive breeding program (USFWS, 1995); however, the latter necessitates that live specimens are collected. Detection of Alabama sturgeon and Gulf sturgeon using eDNA can be used to infer priority localities to concentrate traditional sampling effort which would aid in both conservation goals. In addition, one of the obstacles to sturgeon recovery on the gulf coast includes physical barriers to historical fresh water spawning grounds (USFWS, 1995). Our results demonstrated

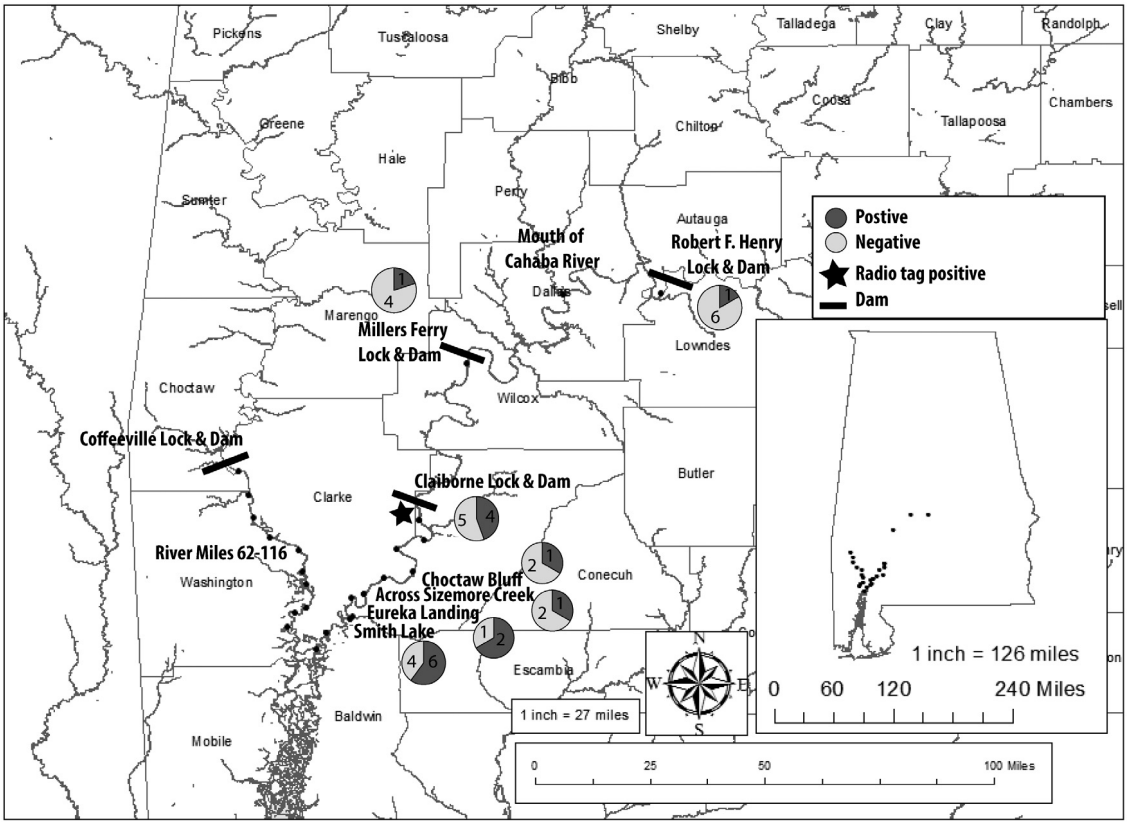


**Fig. 6.** Map of all positive sample locations of Alabama sturgeon using eDNA from April, May and July of 2015. Black circles represent localities sampled. Pie charts indicate both positive and negative samples taken at specific locations. Dark gray indicates positives, while light tray indicates negatives. Lines indicate dams. The star indicates positive radio tag location. Rivers flow north to south.

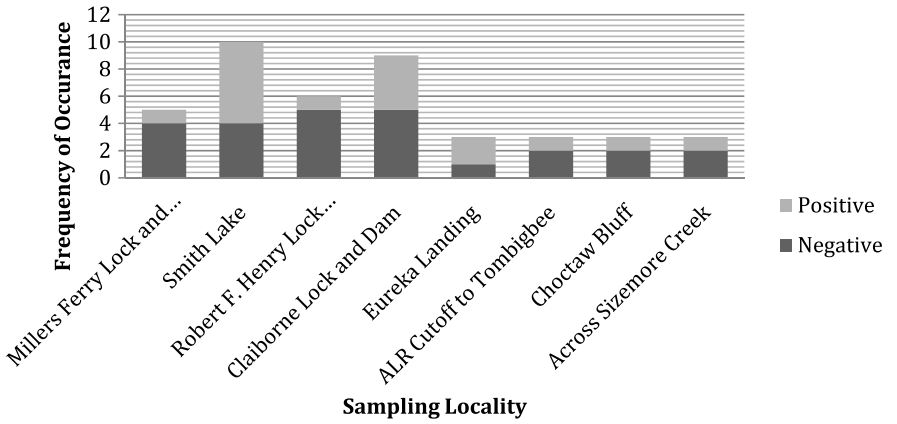


**Fig. 7.** Number of Alabama Sturgeon positive detections by site for April, May and July of 2015. Light gray indicates positive samples, while dark gray indicates negative samples.

6 positive samples for Gulf sturgeon and 2 positive samples for Alabama sturgeon north of Claiborne Lock and Dam, 3 of which for Gulf sturgeon and 1 of which for Alabama sturgeon were also above Millers Ferry Lock and Dam. These results could potentially help to inform and provide additional recovery and management strategies (i.e., fish passage) for these species such as removal of at least one passage barrier. Further study is necessary.

Environmental DNA has proven to be an effective tool for detection of rare and/or invasive species in streams and rivers (Gu and Swihart, 2004; Darling and Mahon, 2011; Goldberg et al., 2011; Jerde et al., 2011; Thomsen et al., 2012a,b; Mahon et al., 2013; Díaz-Ferguson, 2014; Janosik and Johnston, 2015; Boothroyd et al., 2016). This proves to be true with detection of

Alabama and Gulf sturgeon. Higher detection rates using eDNA were accomplished in a short period of time, while traditional sampling can take several years before positive detection is achieved for these species. DNA persists in aquatic ecosystems from 0.9 to 54 days depending on a number of biotic, abiotic, and DNA characteristics (Barnes and Turner, 2016). Thus detection of Alabama and Gulf sturgeon is likely from recent DNA rather than long-term persistence. DNA transfer by vectors (Shaw et al., 2016) such as predators and wetland birds is unlikely for large fish such as the Alabama and Gulf sturgeon. However, DNA transfer from boats and water currents is a possibility. However, given the lack of positives at each site over the temporal scale indicates DNA transfer from vectors and long term persistence is unlikely.

Future directions include expansion of temporal sampling in attempt to reconstruct fish migration up and down the rivers and to locate spawning sites.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.gecco.2016.08.008.

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