Current Distribution and Habitat Use of the Threatened Snail Darter (*Percina tanasi*) in Alabama

by

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Abstract

The distribution of many fishes in larger rivers is poorly known, in part due to the difficulties of sampling them. This is especially true for small -bodied or rare species, such as the Snail Darter (*Percina tanasi)*. This federally listed (threatened) species of darter has a limited distribution in the Tennessee River system in Alabama and Tennessee, where it is known from a few large tributaries or small rivers. The Snail Darter was previously known from only one locality in Alabama, but has recently been found in two additional, widely separated systems. These new distributional records raise questions regarding the accuracy of our current understanding of the range of this species. In particular, are Snail Darters present throughout the main stem Tennessee River, and is the species dispersing into new areas from source populations in the river? The occurrence of Snail Darters in the Tennessee River main stem would expand our knowledge of its range, and inform current conservation efforts for the species, such as habitat prioritization. To clarify the distribution of Snail Darter in Alabama, 61 unique sites were surveyed using environmental DNA for detection. This cost-effective detection tool eliminates the difficulty associated with empirically sampling large rivers for small fishes. Approximately 50% of sites sampled were positive for Snail Darter DNA. This study confirmed the known localities of Snail Darters in the Bear Creek, Elk River, and Paint Rock River. Several new localities were also discovered throughout the main stem Tennessee River and in Shoal Creek, near Florence, Al. Side scan sonar techniques were applied to compare habitat availability

surrounding select negative and positive eDNA sites. Negative sites had a significantly higher proportion of fine sediment compared to the positive sites. These results determined critical localities and habitat types that sustain Snail Darter populations. These findings can inform biologists about where to prioritize conservation efforts and further, could lead to studies assessing movement and relatedness between populations in this system.

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List of Abbreviations

BLAST	Basic Local Alignment Search Tool
eDNA	Environmental DNA
ESA	Endangered Species Act
GPS	Global Positioning System
MMU	Minimum Mapping Unit
PCR	Polymerase Chain Reaction
TVA	Tennessee Valley Authority
UWF	University of West Florida

Introduction

The southeastern United States is home to some of the world's most diverse aquatic communites. More than 450 fish species can be found within the borders of Alabama alone (Boschung & Mayden, 2004). The Alabama portion of the Tennessee River system is home to 178 species, and among these are many endemics that are being threatened with extirpation. Habitat in the Tennessee River has been heavily altered by the construction of dams, often so closely spaced that the tailwaters of one dam lead directly into the headwaters of the next reservoir (Neves & Angermeier, 1990). This habitat alteration has increased the need for the monitoring and conservation of fishes in these rivers.

Effective conservation of threatened species requires the ability to confidently detect where species exist. Imperiled species are often extremely rare and hard to find using traditional sampling methods, such as backpack electrofishing and seining. Detection rates of rare species can be low even in places where they are known to exist. It is even more difficult in situations where a species is dispersing into new localities in low numbers, which may be the case with the Snail Darter (*Percina tanasi).* The Snail Darter is a threatened species endemic to the Tennessee River drainage in Alabama and Tennessee. Recent surveys have identified Snail Darters outside of their known range in Alabama, suggesting that knowledge of the current range of the species may be inaccurate.

The Snail Darter was first discovered in 1973 in the Little Tennessee River in a portion of the river which was proposed for impoundment by the Tennessee River Authority (Etnier, 1976). At the time, this was only location where the Snail Darter was known to exist, and therefore it was listed as endangered under the recently established Endangered Species Act (ESA, 1973).

In 1979, the completion of the Tellico Dam on the Little Tennessee River, threatened the existence of the Snail Darter. Recovery plans shifted from protecting the known habitat of Snail Darters to transplanting populations to suitable habitats in other Tennessee River tributaries (Biggins, et al., 1983). The localities selected for Snail Darter transplants included the Hiwassee, Nolichucky and Holston Rivers in Tennessee, and the Elk River in Alabama. In addition to the relocation into these rivers, other populations were discovered in the Paint Rock River in Alabama, as well as the Sequatchie River, Sewee Creek, and South Chickamauga creek in Tennessee (Biggins, et al., 1983). Since then, new populations were also discovered in the French Broad, Little, and Ocoee Rivers in Tennessee (Service, 2013). This led the Snail Darter to be down listed to threatened in 1983.

The Snail Darter belongs to the subgenus *Imostoma,* which includes the Saddleback Darter (*Percina vigil).* The Saddleback Darter can also be found in the Tennessee River drainage in Alabama and is thought to be sympatric with the Snail Darter (Boschung & Mayden, 2004). Like all members of *Imostoma,* Snail Darters inhabit rivers and larger creeks preferring sand and gravel shoal areas with high flow. Ashton and Layzer (2010) found that they typically avoid silt-covered substrates and preferred clean substrates when feeding. Snail Darters have a distinctive dorsal color pattern of four dark saddles providing excellent camouflage on sand and gravel substrates (Etnier & Starnes, 1993). They have also been found to occur in deeper portions of rivers and reservoirs, but it is not known to what extent they utilize the impounded areas in the Tennessee River reservoirs. Snail Darters also have the ability to burrow beneath the substrate, likely for further concealment purposes.

Snail Darters are a small bodied and relatively short-lived species, with few individuals surviving into a fourth year and ranging in size from 52-85mm (Starnes, 1977). Spawning takes place early in the year from February to mid-April. Snail Darter larvae will drift in the water for 15-20 days after hatching, ultimately seeking refuge in slow moving, pool habitat (Etnier & Starnes, 1993). This larval drift is thought to be a mechanism for downstream dispersal (Service, 2013). Snail Darter attraction to current as they mature can possibly lead them to other streams with appropriate habitat and water quality.

Recent studies on Snail Darter distribution have established that the largest extant populations of Snail Darters occur in the French Broad and Hiwassee Rivers (Ashton & Layzer, 2008). They are also thought to occupy the riverine portions of five main stem reservoirs in the upper Tennessee River (Service, 2013). Until recently, Snail Darter distribution in Alabama was thought to solely consist of a small, yet stable population in the Paint Rock River. It was believed that the Elk River transplant population did not succeed (Etnier & Starnes, 1993). However, in 2015, individuals were collected at two localities in Bear Creek and in the Elk River. Bear Creek is one of the most well sampled system in Alabama, so it is unlikely that a Snail Darter population has been historically present there without detection. Alternatively, Snail Darters could have recently entered Bear Creek via the Tennessee River. A population reemerging in Elk River is also interesting due to it being more than 30 years after the transplant population was deemed unsuccessful. Either a small population persisted without detection, or the species has also recently entered via the Tennessee River. These discoveries raise many questions, as not only is that a very far distance to travel in both cases, but in the case of Bear Creek, the Wheeler

and Wilson Dams separate the that population from the only previously known population in the Paint Rock River.

Thus, traditional survey methods alone are too unreliable to definitively say where the Snail Darter exists today, as Snail Darter are either too rare or currently dispersing into systems where they are not known to occur. A relatively new technique for validating the presence of such species is environmental DNA, referred to as eDNA. This is a forensic technique that allows for the detection of species without any direct contact. Scales, slime, gametes, and feces, which contain DNA, can be collected in simple water samples if the species is present in the system (Janosik & Johnston, 2015; Jerde et al., 2011; Rees et al., 2014).

DNA degrades rather quickly in the environment and becomes harder to detect over time due to a variety of factors. Recent studies examining eDNA persistence in water have shown varying results. In one study, eDNA was undetectable after just one day in a marine aquarium (Thomsen et al., 2012). Another study was still able to detect eDNA after 25 days in a lab controlled environment (Dejean et al., 2011). However this is likely an extreme case as eDNA decays exponentially and the detection probability has been found to be less than 5% after four days (Barnes et al., 2014). Probability of detection is also influenced by the transport distance of eDNA material. In larger systems, as DNA material moves downstream it can become diluted where detection can become less likely (Jane et al., 2015). In headwater streams, eDNA has been found to be detectable at distances of 50 meters up to 240 meters (Jane et al., 2015; Wilcox et al., 2016).

Environmental DNA can play a huge role in effectively managing aquatic systems. For example, it has been used to detect the invasion of harmful species earlier than previously

possible which increases the feasibility of eradicating or containing the invader (Goldberg et al., 2013; Jerde et al., 2011; Wilcox et al., 2016). eDNA also serves as a great tool for detecting hard to find and imperiled species. This method was recently used with great success to detect the extremely rare Alabama Sturgeon in the Alabama River. The Alabama sturgeon was considered virtually undetectable using standard methods due to low numbers and migratory behavior in large river systems. Environmental DNA was able to confidently determine that the Alabama Sturgeon is still extant in several locations with much more effectiveness than traditional methods (Pfleger et al., 2016).

Another recent study by Janosik & Johnston (2015) showed that when compared to traditional methods, eDNA was vastly more effective at detecting a rare species. The imperiled Slackwater Darter was only found in one out of the 49 sites using traditional methods compared to 23 positive detections using eDNA (Janosik & Johnston, 2015). Further, this technique has been used and empirically tested in several other studies to detect aquatic species. This technique has been coupled with quantitative fisheries sampling and demonstrated that eDNA provided detection when conventional sampling did not (Goldberg et al., 2011; Thomsen et al., 2012; Jerde et al., 2011). Detecting where invasive or listed species exist is often the first step in being able to protect critical habitat and can help further understand how fishes move throughout river systems.

The current distribution of the Snail Darter in Alabama is relatively unknown. Aside from a historically known population in the Paint Rock River and the recent discoveries in Bear Creek and Elk River, questions remain about the extent of their distribution. The goal of this study was to use environmental DNA in conjunction with empirical sampling methods to help determine

the distribution of the Snail Darter throughout the Tennessee River system in northern Alabama. This research will attempt to answer three primary questions:

1. Where are Snail Darters utilizing main stem Tennessee River habitat?
2. Which tributaries are they occupying?
3. What substrate types are Snail Darters utilizing/avoiding in the main stem river?

Snail Darter status in the main stem of the Tennessee River has not been assessed since 1984, and when the recovery plan was finalized it was thought that they did not occupy impounded main stem reaches (Service, 2013). Historically, their occupancy throughout the main stem river has been difficult to determine. Snail Darter occupy large riverine systems, and they are thought to exist in low numbers where they are present (Ashton & Layzer, 2008). This results in a very low catch probability with regards to traditional sampling methods. The Alabama portion of the Tennessee River has several major tributaries. In addition to the recently discovered populations in Bear Creek and the Elk River and the historically known population in the Paint Rock River, it is possible that Snail Darter populations have avoided detection in other tributaries as well, such as Shoal Creek and the Flint River.

Materials and Methods

Field Sampling and Study Area

70 water samples were collected throughout the Tennessee River system in Northern Alabama in 2017 and 2018. Specifically, 40 samples were collected from the main stem Tennessee River, 11 samples were collected from the Bear Creek system, nine samples were collected from the Paint Rock River, five samples were collected from the Elk River, four samples were collected from the Flint River, and two samples were collected from Shoal Creek.

Nine of the above locations were sampled twice. The environmental DNA collection procedure was similar to Ficetola et al. (2008) and Thomsen et al. (2012). Three replicates of 15 mL water samples were collected at each sample site in the same location. The sample was collected from the surface of the water. Upon collection, 1.5 mL of 3M sodium acetate and 33 mL of 95% ethanol were added to each 15ml replicate. Water samples were transported in coolers and stored at room temperature until DNA extraction. A control consisting of the 3M sodium acetate, ethanol, and 15 ml of deionized water was included in the cooler to ensure no contamination occurred during collection and transportation during all sampling trips.

DNA Extraction

Extraction of DNA was completed using the DNeasy® Blood and Tissue Kit (Qiagen, Inc., Valencia, CA). All water samples were first centrifuged for 30 minutes at 4°C at 3500 RPM. The supernatant was then discarded and the pellet and remaining ethanol were transferred to a 1.5 mL tube with a sterile pipette and centrifuged at 14000 RPM for 3 minutes. The DNA extraction followed DNeasy® spin column protocol. The three replicate water samples from each site were pooled into one sample. Extraction controls were included to ensure contamination did not occur during the DNA extraction process.

Primer Design

To design species-specific primers, genetic resources for Snail Darter were collected from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Sequences were downloaded and aligned using Geneious 8.0 (http://www.geneious.com/). A small, 153-base pair segment of the cytochrome-*b* subunit of mitochondrial DNA was targeted. Primers (L: 5’ CCCTATTCGCCCCTAACCTA 3’; R: 5’ GGGCTAAAACACCCCCTAGT 3’) were designed

using Primer 3 (Untergasser et al., 2012) and Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast). These primers were compared against available sequence data from other closely related species potentially present in the system (*Percina vigil* and *Percina shumardi).* The primers were further tested with tissue derived DNA from Saddleback and Snail Darters to ensure amplification of only the target species.

PCR and Sequencing

All water samples were run through the OneStep™ Inhibitor Kit (ZYMO RESEARCH). Polymerase Chain Reaction (PCR) was used for amplification. Samples were analyzed by PCR amplification methods using 2.5µL of DreamTaq™ (10X, ThermoFisher Scientific) buffer, 0.5µL of dNTP, 1 µL of each forward and reverse primer, 0.3 µL of DreamTaq™ (ThermoFisher Scientific), 18.7µL of Milli-Q® H_2O, and 1µL of extracted DNA from the sample for a total reaction volume of 25 µL. The PCR conditions were as follows: initial incubation at 94 °C for 3 min, followed by 30 cycles of 95 °C for 30 seconds, 55.8 °C for 30 seconds, 72 °C for 1 min and 30 seconds, with a final extension at 72 °C for 5 min. The PCR results were visualized using electrophoresis on agarose gel stained with ethidium bromide.

Each sample was independently run three times to reduce the risk of false negatives and to increase the probability of positive detections (Yamamoto et al., 2017) (Ficetola et al., 2015) . Select positive samples were purified for sequencing using Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (ThermoFisher Scientific) to ensure species specificity and bidirectionally sequenced. Sequences were edited using Sequencher 5.4.5 (Gene Codes Corporation, Ann Arbor, MI, USA) and confirmed using nucleotide BLAST (Basic Local Alignment Search Tool, blast.ncbi.nlm.nih.gov). Confirmed positive detections of Snail Darter

DNA indicate that the species was present in the river at that location. Due to relatively rapid DNA degradation, dilution, limited transport distance, and likely low density of individuals, any positive eDNA sample suggests it originated in close proximity to the collection site.

Side-Scan Sonar

Sonar surveys were conducted surrounding ten main stem river sampling sites in September 2018. These sites included five negative and five positive eDNA locations, primarily in more riverine sections and the areas below the Wilson and Guntersville Dams. These areas were relatively shallow and were the least wide sections of the river which allowed for effective sonar surveys. A Humminbird® 997c SI combo system was used to acquire sonar imagery with a bow mounted transducer. Each segment consisted of four to seven parallel passes moving downstream at approximately four miles per hour following a GPS track to ensure complete coverage of the river. Each pass had total sonar beam width of 106 meters with a frequency of 455 kHz.

The sonar image and GPS track files were processed using the program SonarTRX (LEI 2017), subsequently imported into ArcMap 10.6.1 (ESRI 2018), and projected using WGS1985 UTM Zone 15N. Four coarse substrate types were established (Table 2) and polygons were digitized over these substrate patches seen on the sonar image. A minimum mapping unit (MMU) of 500m^2 was determined. Boundaries were digitized around all substrate patches ≥MMU and then converted into polygons with a known area.

To account for drifting eDNA and fish movement and to better compare between sampling sites, a subsection consisting of a semi-circle with a 200-meter radius was cut out from the digitized substrate map. Proportions of substrate available in each semi-circle beginning

immediately upstream of each sampling site were them analyzed between negative and positive locations. Due to a low sample size of ten total sites, and the non-normally distributed data, a Kruskal-Wallis non-parametric test (Kruskal & Wallis, 1952) was used to compare the substrate proportions between the negative and positive sites.

Results

Snail Darter DNA was detected at 30/61 (49%) sites sampled in the Tennessee River system (Figure 1). Positive eDNA detections include stream systems where Snail Darters were previously detected (Bear Creek, Elk and Paint Rock rivers), as well as from sites throughout the Tennessee River main-stem where they have not been previously documented. Additionally, Snail Darter DNA was detected in Shoal Creek, where they have never been documented.

Of the 40 sites sampled in the main stem river, 20 (50%) were positive for Snail Darter DNA. These positive sites are mainly found in the more riverine sections of the river, with the exception of three positives found in the more lentic section of Pickwick Reservoir near the mouth of Bear Creek.

Snail Darter DNA was obtained in five of the seven sites in the Bear Creek system including Bear, Cedar, and Rock Creeks. These positive sites include the two localities where individuals were collected in 2015. The Goose Shoals site in Shoal Creek was sampled on two separate dates and returned positive for Snail Darter both times. Five sites were sampled in the Elk River and two of these were positive for Snail Darter DNA. The Elk River findings follow the same trend as the river samples as the positive sites were found in faster flowing shoal habitats and the negatives were found further downstream where the habitat is more lentic where the Elk river is backed up from Wheeler Reservoir. Snail Darter DNA was detected in the Paint Rock River in

only one of the six locations that were sampled. Snail Darter DNA was not found in the Flint River (0/4). No positive signatures were detected in collection, extraction, or PCR controls.

The average substrate composition surrounding the five negative eDNA sites consisted of 78.74% fine sediment, 6.32% large boulder, 13.96% gravel/cobble, and 0.99% bedrock (Figures 7 & 9). The five positive eDNA sites consisted of 30.92% fine sediment, 23.14% larger boulder, 25.42% gravel/cobble, and 20.52% bedrock (Figures 8 & 9). Positive eDNA sites had significantly less fine sediment when compared to the negative eDNA sites (Kruskal-Wallis non-parametric test: $H = 3.94$, $df = 1$, $P = 0.0472$). There were no significant differences among the other three substrate types.

Discussion

The Snail Darter is more widely distributed in Alabama than previously thought. Their distribution does not seem to be limited to certain segments of the river and is found throughout the entirety of the system in Alabama. With the exception of three positive sites in close proximity to the mouth of Bear Creek in Pickwick Reservoir, the positive sites were found in the more free-flowing sections of the river and populations do not appear to be utilizing the more impounded sections. These data cannot directly inform whether these newly discovered populations are the result of recent a long-range dispersal event or an indication of increasingly detectable population numbers. However, the widespread distribution throughout the main stem river and occupancy in several tributaries may suggest that the recent collections in Bear Creek and Elk River are the result of a recent invasion via main stem river populations.

Recent dispersal of Snail Darters could have been enabled by improved dam management practices implemented in the 1990s by the Tennessee Valley Authority (TVA). In the 1970s and

80s, when the last main stem river surveys for the Snail Darter were conducted, environmental conditions below the dams were very poor (Bednarek & Hart, 2005). During periods of non-generation, water depths and velocities in the sections below the dam were at extremely low levels. Also, these dams released water with very low dissolved oxygen levels. These factors were improved with new management practices that defined minimum flows and dissolved oxygen targets to improve water quality and habitat below the dams (Higgins & Brock, 1999). These new management practices led to a significant change in macroinvertebrate assemblage, with increasing distance below the dams and with family richness increasing and percent tolerant species decreasing (Bednarek & Hart, 2005). Fish communities likely benefited from these management and habitat improvements as well, and this may have allowed the Snail Darter to occupy previously uninhabitable reaches in the main stem river.

Minimum flow practices enacted during the spring would have a large impact on the Snail Darters ability to disperse into new localities. This could have led to increased survival and recruitment of Snail Darters and also allowed for larval Snail Darters to drift further downstream. It is believed that the French Broad River population originated from the larval drift of spawning Snail Darters in the Holston River (Service, 2013). In this case, larval Snail Darters would have drifted 14.7 miles downstream in the Tennessee River to reach the mouth of the French Broad River. This phenomenon over time could have led to the most downstream population currently known of today in Bear Creek.

An examination of the substrate data surrounding select positive and negative eDNA sites suggest that Snail Darter prefer the rockier substrate in the Tennessee River rather than sediment dominated areas. In particular, the Wilson and Guntersville Dam tailraces seem to provide

important habitat for this species, which are flowing, rocky shoal areas of the river. Snail Darters utilize these gravel shoal areas for feeding and reproduction and fine sediment degrades spawning habitat and limits food availability (Ashton & Layzer, 2008). One of the largest threats to Snail Darter populations is increased sedimentation. Many of the major tributaries of the Tennessee River flow through agricultural lands, which can lead to sediment loading during periods with high runoff. Also, barges in the main stem river contribute to increased turbidity in the water column and stream bank erosion (Service, 2013). These factors could lead to the restriction of the available habitat and overall distribution of the Snail Darter.

This study has major conservation implications in regard to the new populations identified. Imperiled species need protection and it is impossible to protect species if their presence is unknown. Information gleaned from this study furthers knowledge about a species that surprisingly has many gaps, especially in regard to main stem Tennessee River use. This is the first applied use of eDNA on the Snail Darter and the developed tools can be used in the future to continually monitor the distribution and dispersal of this species. With this widespread distribution, it is possible that other undiscovered tributary and main stem populations exist both in Alabama and Tennessee.

Comprehensive eDNA studies should be conducted in the future to fully understand the distribution of the Snail Darter. These newly discovered Snail Darter localities will allow for targeted empirical sampling efforts in areas with higher chances of capture success, eventually leading to further studies on population dynamics, abundance, and reproduction of these previously unknown populations. Further studies should also examine phylogeographic relationships to assess whether these populations are genetically isolated or the result of dispersal

from upstream populations. Stable reproducing populations in the main stem river in addition to the expansion of viable tributary populations could eventually lead to delisting of the Snail Darter as a threatened species, as the above would meet the criteria established in the Snail Darter recovery plan (Biggins et al., 1983). Efforts to improve dam operations and minimize sedimentation should continue to ensure the recovery of Snail Darter populations. Small bodied fishes in large river systems often evade detection (Herzog et al., 2009) and this study has shown that eDNA can be a highly effective tool in these environments. eDNA should be considered in future studies evaluating species distribution and movement in large river systems where traditional sampling would be ineffective.

Tables and Figures

Table 1. Environmental DNA collection site appendix and results

River	County	Site #	Site Description	LAT (DD)	LONG (DD)	Date	eDNA Result
Bear Creek	Tishomingo (MS)	1	HWY 30 Bridge	34.63410	-88.15448	9/21/17	NEG
Bear Creek	Tishomingo (MS)	1	HWY 30 Bridge	34.63410	-88.15448	6/11/18	POS
Cedar Creek	Colbert	2	Natchez Trace Pkwy	34.64463	-88.13260	9/21/17	NEG
Cedar Creek	Colbert	2	Natchez Trace Pkwy	34.64430	-88.13260	7/25/17	POS
Rock Creek	Colbert	3	Maud Rd.	34.63220	-88.09020	7/25/17	POS
Rock Creek	Colbert	4	Natchez Trace Pkwy	34.65710	-88.09530	7/25/17	NEG
Rock Creek	Colbert	4	Natchez Trace Pkwy	34.65710	-88.09530	9/21/17	NEG
Bear Creek	Colbert	5	Bear Creek Picnic Area	34.67428	-88.08878	3/15/18	POS
Bear Creek	Colbert	5	Bear Creek Picnic Area	34.67428	-88.08878	6/11/18	POS
Bear Creek	Colbert	6	County Rd. 1	34.73448	-88.08911	7/25/17	POS
Bear Creek	Colbert	7	County Rd. 1 Reservoir	34.76570	-88.08500	7/25/17	NEG
Tennessee River	Colbert	8	Mouth of Bear Creek	34.87880	-88.09430	7/11/17	POS
Tennessee River	Colbert	9	D.S. Bear Creek RM 225	34.89247	-88.09983	6/12/18	POS
Tennessee River	Lauderdale	10		34.90090	-88.06490	7/11/17	POS
Tennessee River	Lauderdale	11		34.84560	-87.94110	7/11/17	NEG
Tennessee River	Lauderdale	12		34.79040	-87.89800	7/11/17	NEG

Tennessee River	Colbert	13	D.S. Cane Creek RM 244	34.75854	-87.85848	6/12/18	POS
Tennessee River	Lauderdale	14		34.73810	-87.81720	7/11/17	NEG
Tennessee River	Colbert	15	Buck Island Complex	34.73396	-87.77722	6/12/18	POS
Tennessee River	Lauderdale	16	Pippin Toe Head RM 250	34.74059	-87.76940	6/12/18	NEG
Tennessee River	Lauderdale	16	Pippin Toe Head RM 251	34.74059	-87.76940	7/11/17	NEG
Tennessee River	Lauderdale	17	7-mile island RM 252	34.75657	-87.73545	6/12/18	POS
Tennessee River	Lauderdale	18		34.76660	-87.71820	7/11/17	POS
Tennessee River	Lauderdale	19	D.S. Cypress Creek RM 255	34.77759	-87.69665	6/12/18	POS
Tennessee River	Lauderdale	20	D.S. HWY 72 bridge RM 259	34.78320	-87.67316	6/12/18	POS
Tennessee River	Lauderdale	21		34.78650	-87.66030	7/11/17	NEG
Tennessee River	Lauderdale	22	D.S. Wilson Dam RM 256	34.79532	-87.62942	6/12/18	POS
Shoal Creek	Lauderdale	23	Goose Shoals	34.95340	-87.59308	3/15/18	POS
Shoal Creek	Lauderdale	23	Goose Shoals	34.95340	-87.59308	6/25/18	POS
Tennessee River	Lauderdale	24	Wilson Lake	34.84268	-87.54100	9/6/18	NEG
Tennessee River	Lawrence	25	Wilson Lake – D.S. Wheeler Dam	34.79642	-87.38869	9/6/18	NEG
Tennessee River	Lauderdale	26	Mouth of Elk River	34.75720	-87.26560	7/11/17	NEG
Elk River	Lauderdale	27		34.80240	-87.23460	7/11/17	NEG
Elk River	Limestone	28	Elk River Mills Rd. Bridge	34.84745	-87.11714	9/21/17	NEG
Elk River	Limestone	29	Easter Ferry Rd. Bridge	34.92263	-87.04903	9/21/17	NEG
Elk River	Limestone	30	Mason Island	34.97439	-87.00585	6/12/18	POS
Elk River	Limestone	31	Upper Shoal	34.98892	-87.00585	6/12/18	POS
Tennessee River	Limestone	32	Wheeler Lake	34.68149	-87.07095	9/6/18	NEG
Tennessee River	Morgan	33	D.S. Flint Creek	34.58354	-86.92653	6/13/18	NEG
Tennessee River	Limestone	34		34.58635	-86.92271	7/11/17	NEG
Tennessee River	Limestone	34	Wheeler Wildlife Refuge	34.58635	-86.92271	6/13/18	POS
Tennessee River	Limestone	35	Near mouth of Limestone Creek	34.57859	-86.88662	6/13/18	POS
Tennessee River	Limestone	36		34.55150	-86.79470	7/11/17	NEG
Tennessee River	Morgan	37	Near mouth of Cotaco Creek	34.55695	-86.74960	6/13/18	POS
Tennessee River	Morgan	38		34.55600	-86.67200	7/11/17	NEG
Tennessee River	Marshall	39	Near Black Bluff	34.51279	-86.53663	6/13/18	POS
Tennessee River	Madison	40		34.50700	-86.53400	7/11/17	NEG
Flint River	Madison	41	Mouth of River	34.50303	-86.52799	6/13/18	NEG
Flint River	Madison	42	County Road 61	34.59314	-86.46796	6/11/18	NEG
Flint River	Madison	43	Old 431, Flint River Greenway	34.65131	-86.44853	3/15/18	NEG
Flint River	Madison	44	HWY 72 Bridge	34.74111	-86.44124	3/15/18	NEG

Tennessee River	Madison	45		34.48000	-86.47500	7/11/17	NEG
Tennessee River	Madison	46	D.S. Paint Rock (Clark Bluff)	34.47780	-86.47119	6/13/18	POS
Paint Rock River	Madison	47	Mouth of River	34.47706	-86.46629	6/13/18	POS
Paint Rock River	Marshall	48	HWY 431 Bridge	34.49924	-86.39122	6/11/18	NEG
Paint Rock River	Marshall	48	HWY 431 Bridge	34.49924	-86.39122	3/15/18	NEG
Paint Rock River	Jackson	49	HWY 72 Bridge	34.62407	-86.30658	6/11/18	NEG
Paint Rock River	Jackson	49	HWY 72 Bridge	34.62407	-86.30658	3/15/18	NEG
Paint Rock River	Jackson	50	Canoe Launch in town of Paint Rock	34.65996	-86.32619	6/11/18	POS
Paint Rock River	Jackson	51	Roy B. Whitaker Preserve	34.67414	-86.31707	6/11/18	NEG
Paint Rock River	Jackson	52	County Road 20	34.75325	-86.23372	6/11/18	NEG
Tennessee River	Marshall	53	D.S. Shoal Creek (Guntersville)	34.43790	-86.42114	6/13/18	NEG
Tennessee River	Marshall	54		34.42700	-86.40100	7/11/17	POS
Tennessee River	Marshall	55	D.S. Guntersville Dam	34.42400	-86.39708	6/13/18	POS
Tennessee River	Marshall	56	Guntersville Lake HWY 431 Bridge	34.37487	-86.29658	9/7/18	NEG
Tennessee River	Marshall	57	Guntersville Lake State Park	34.39913	-86.21732	9/7/18	NEG
Tennessee River	Jackson	58		34.66200	-85.94400	7/12/17	POS
Tennessee River	Jackson	59		34.75700	-85.85600	7/12/17	POS
Tennessee River	Jackson	60		34.87200	-85.75900	7/12/17	POS
Tennessee River	Jackson	61		34.98400	-85.70000	7/12/17	NEG

Table 2. Classification scheme used for the Tennessee River substrate maps

Substrate Class	Description	Habitat Score
Cobble/Gravel	>75% of area composed of rocks >2mm but <500mm in diameter across longest axis	4 (Best)
Boulder	>75% of area composed of >3 adjacent boulders >500mm across longest axis	3
Bedrock	>75% of area composed of exposed bedrock (fractured or smooth)	2

Fine Sediment	>75% of area composed of particles <2mm in diameter (Sand, mud, silt, clay)	1 (Worst)

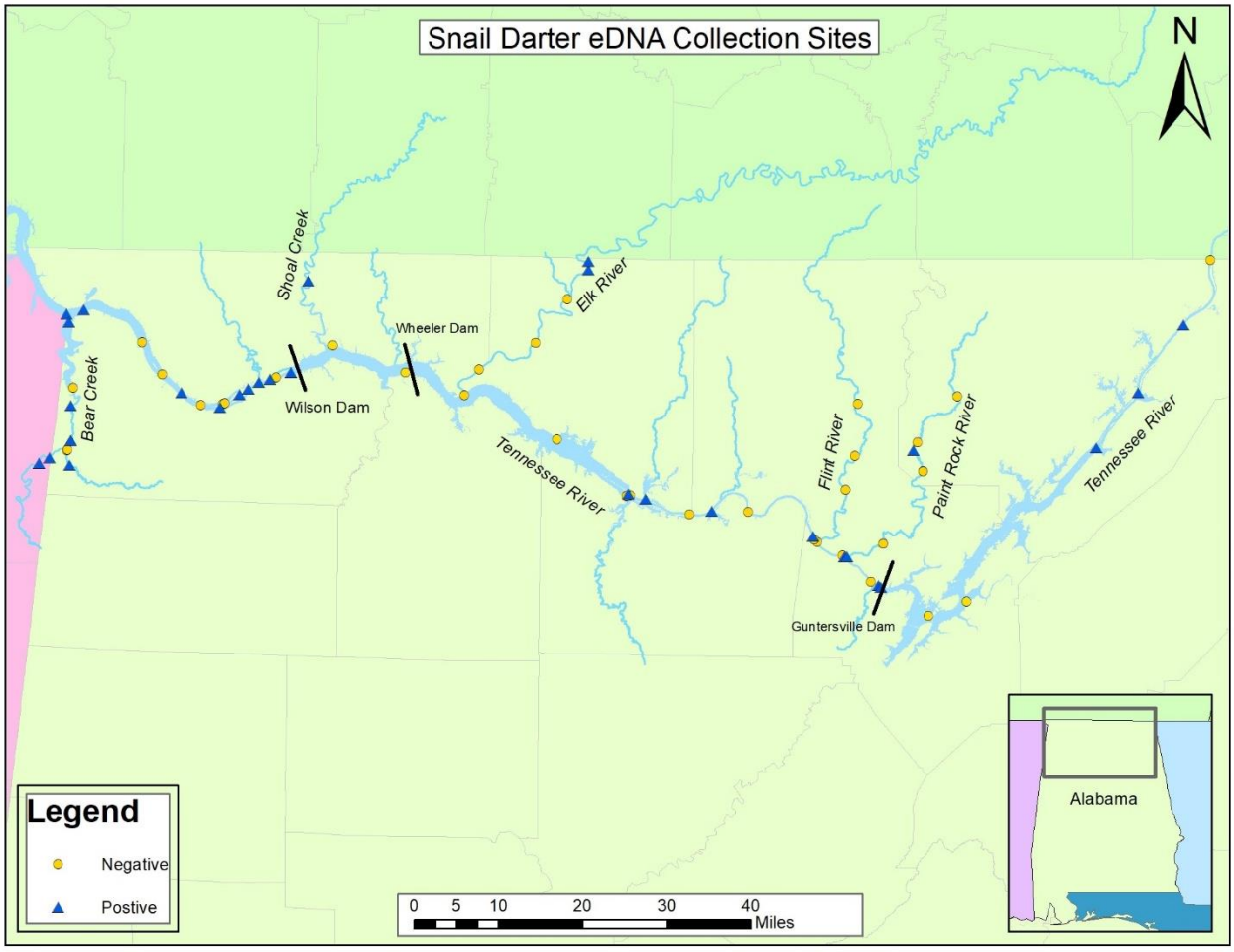

Figure 1. Overview map of eDNA collection sites throughout the Tennessee River system in Northern Alabama. Yellow dots represent the negative sites and blue triangles represent the sites where Snail Darter DNA was obtained.

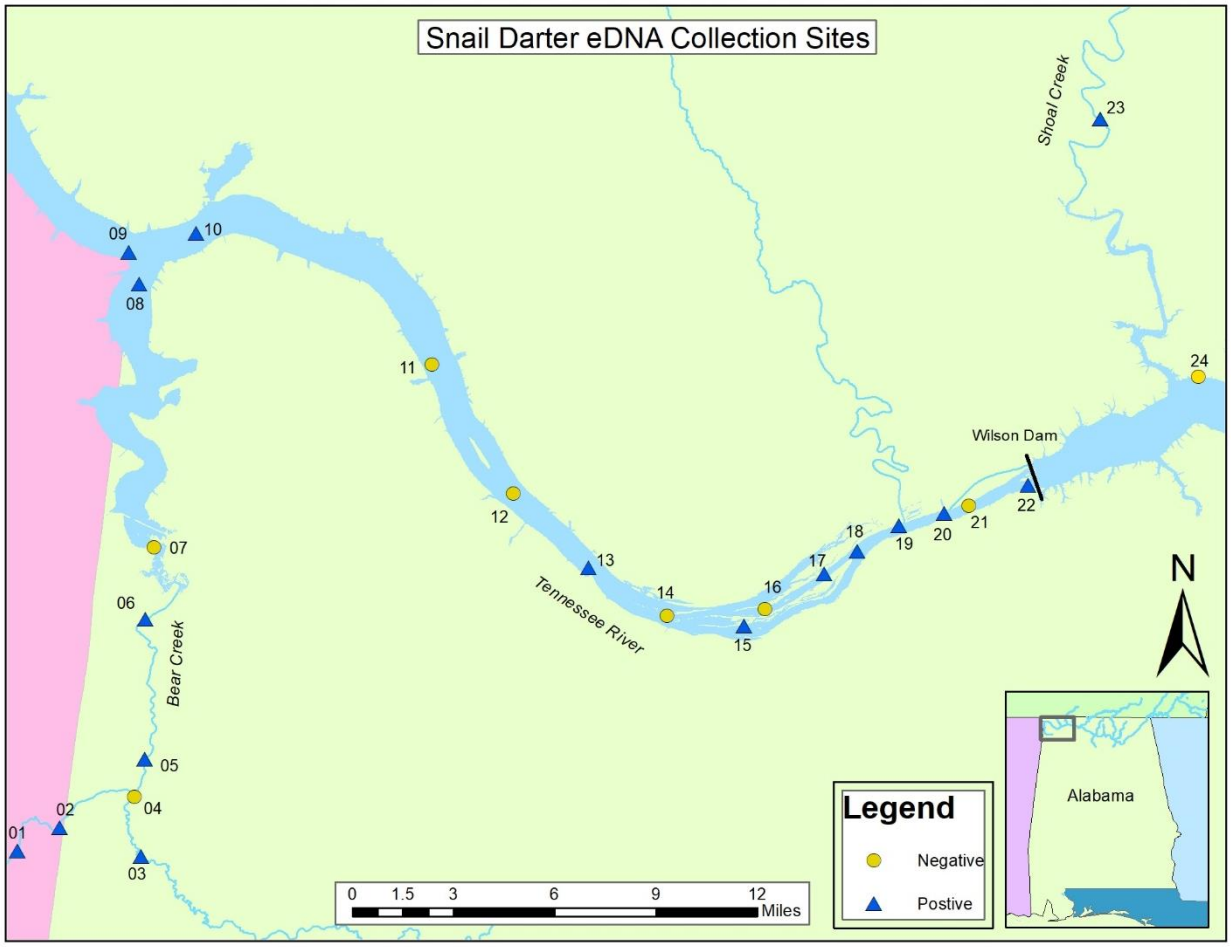

Figure 2. Map of eDNA water collection sites 01-24 in Northwestern Alabama. Yellow dots represent the negative sites and blue triangles represent the sites where Snail Darter DNA was obtained.

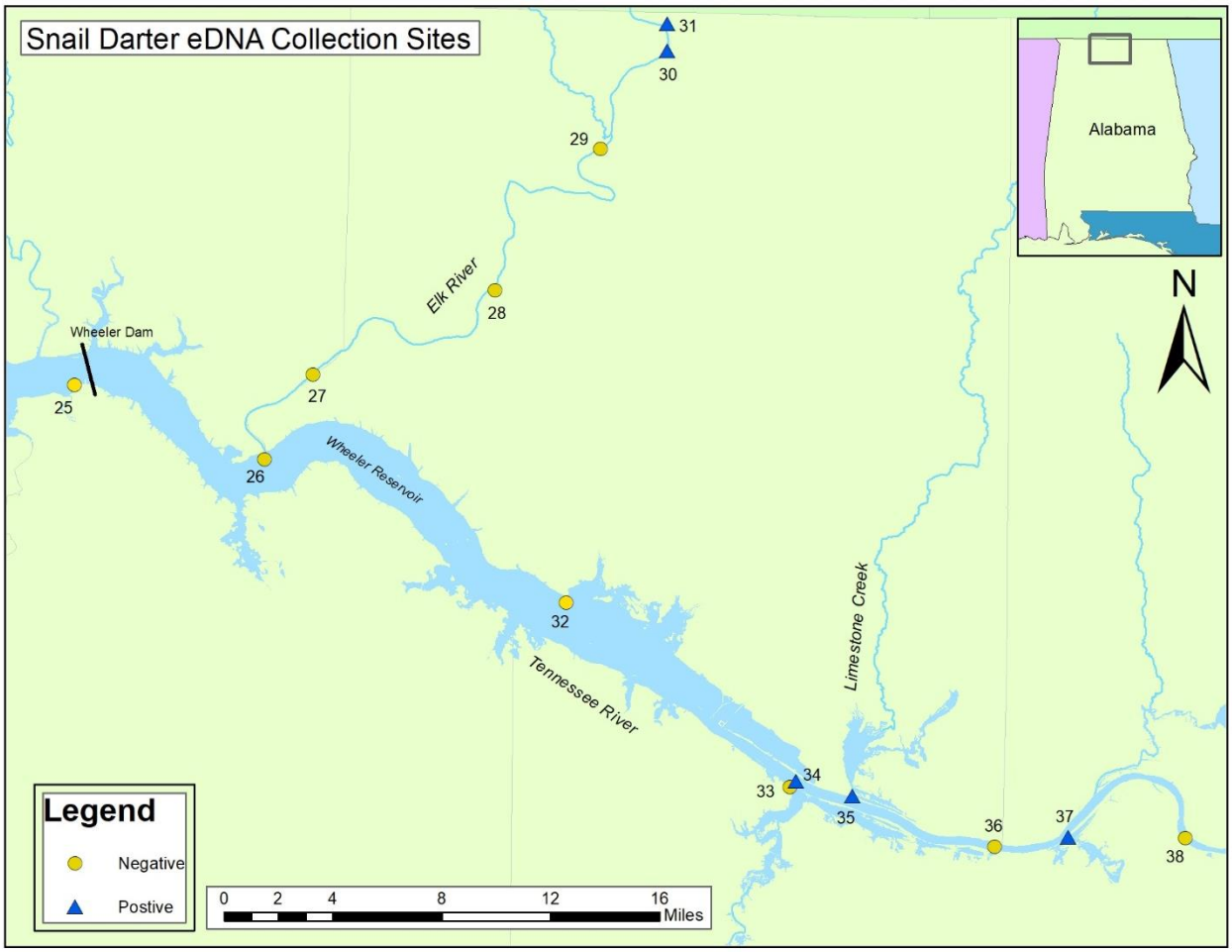

Figure 3. Map of eDNA water collection sites 25-38 in Northcentral Alabama. Yellow dots represent the negative sites and blue triangles represent the sites where Snail Darter DNA was obtained.

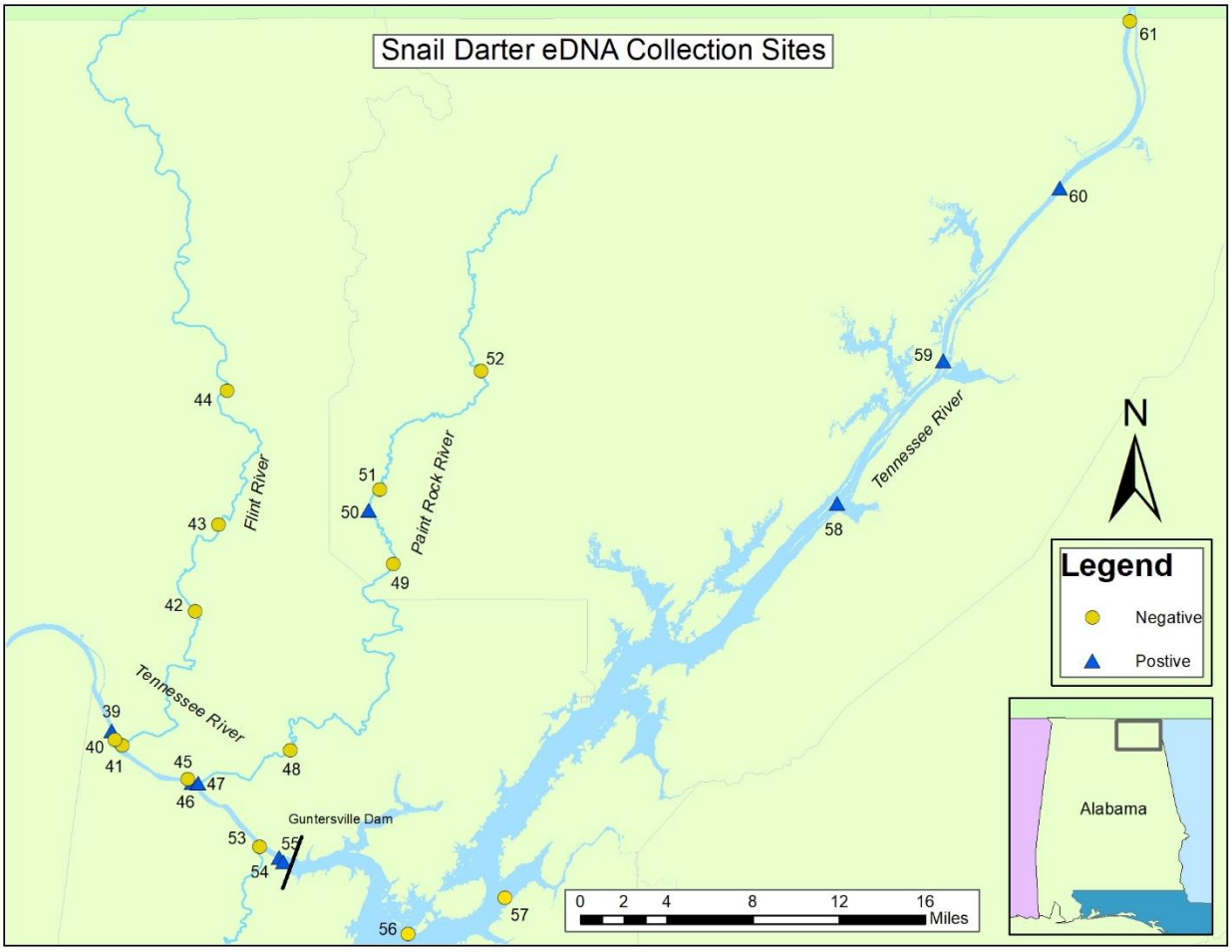

Figure 4. Map of eDNA water collection sites 29-61 in Northeastern Alabama. Yellow dots represent the negative sites and blue triangles represent the sites where Snail Darter DNA was obtained

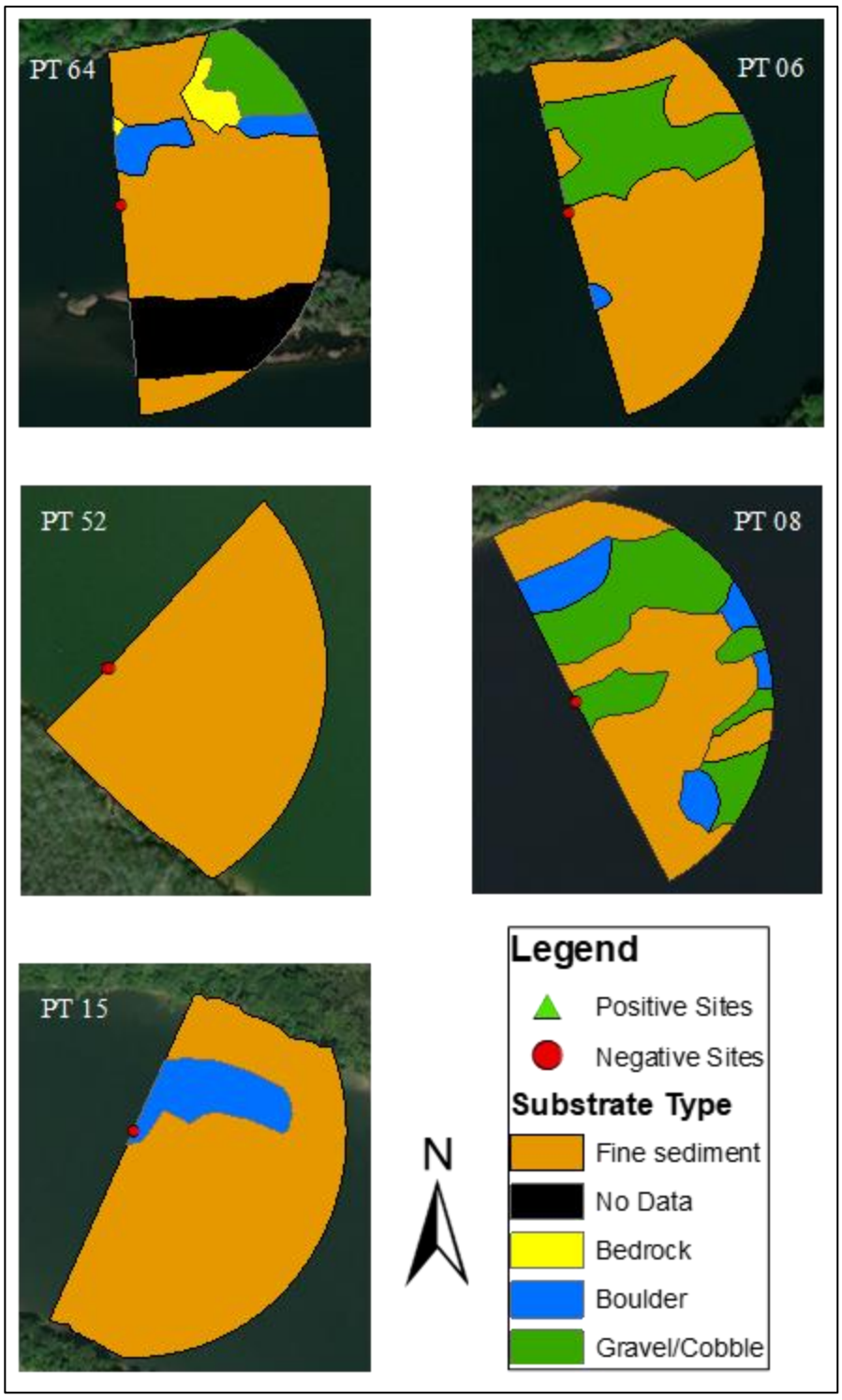

Figure 5. Digitized substrate maps for the negative side scan sonar sites.

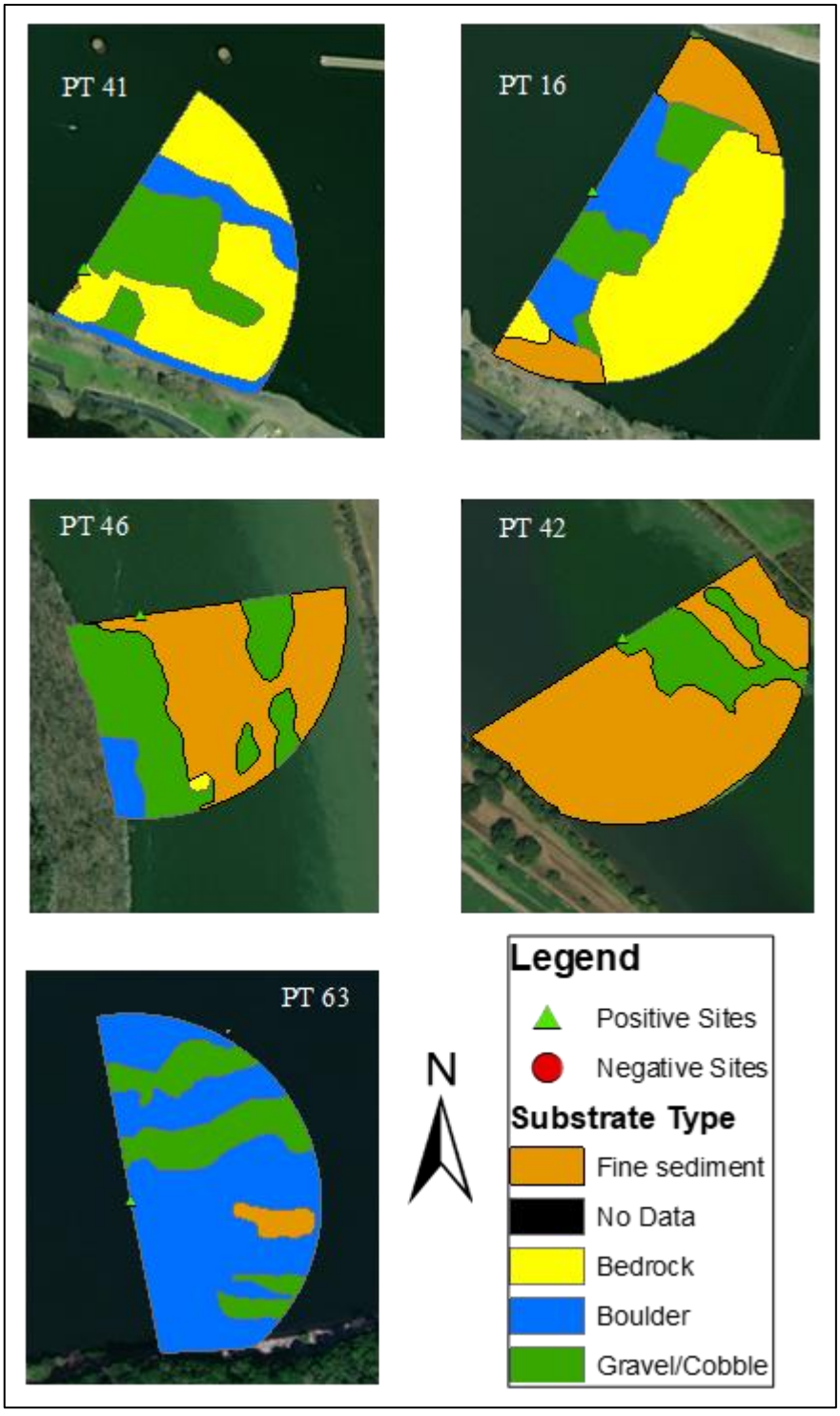

Figure 6. Digitized substrate maps for the postive side scan sonar sites

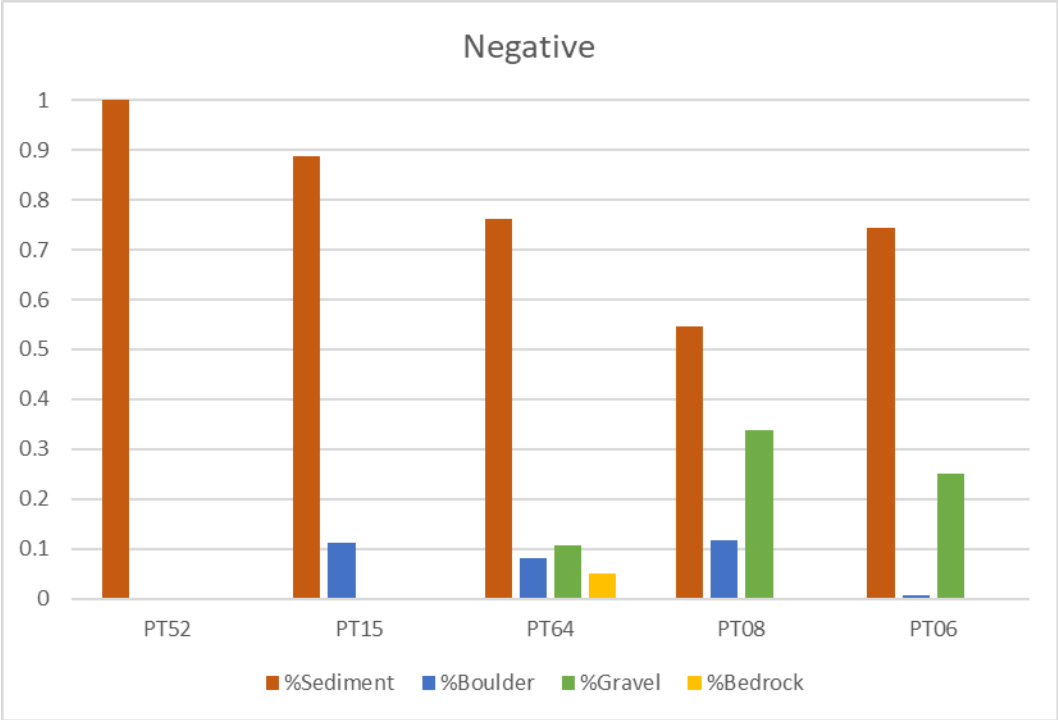

Figure 7. Proportion of substrate around the 5 negative sites that were assessed with Side-Scan sonar.

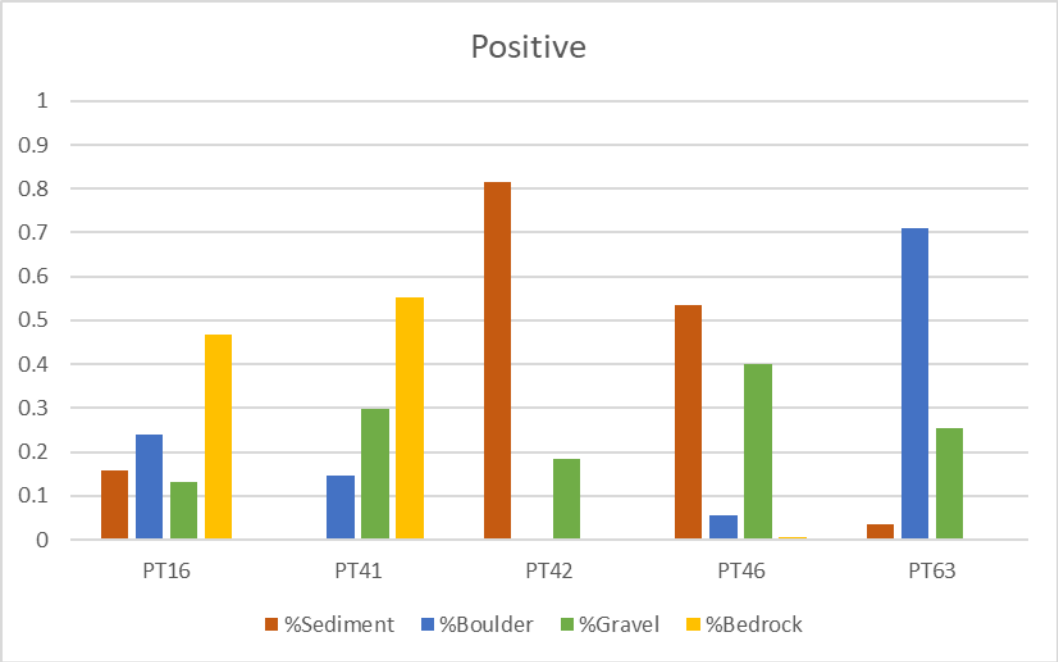

Figure 8. Proportion of substrate around the 5 positive sites that were assessed with Side-Scan sonar.

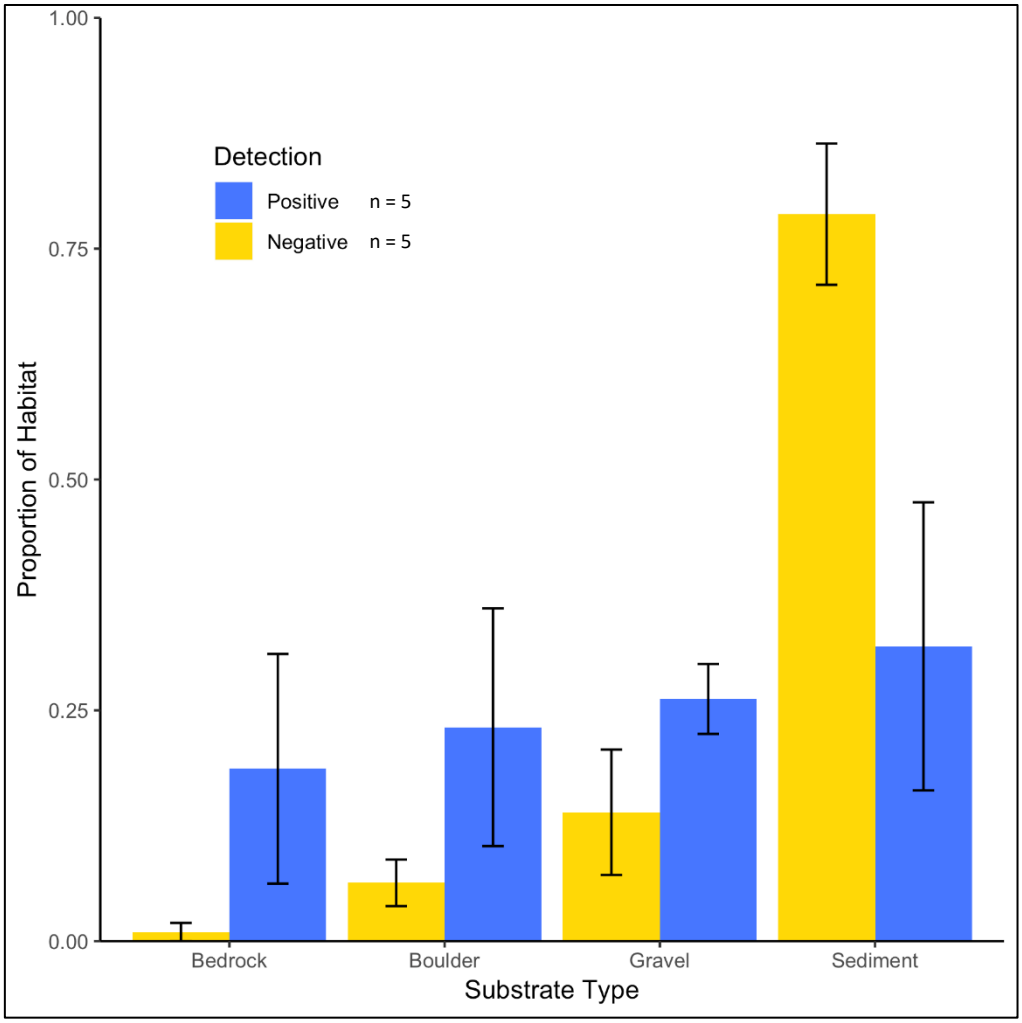

Figure 9. Average proportion of the four substrate types surrounding the negative and positive sites recorded with side-scan sonar

REFERENCES

Ashton, M. J., & Layzer, J. B. (2008). Distribution of the threatened snail darter (Percina tanasi). *Tennessee Academy of Sciences*, *83*(January), 52–56.

Ashton, M. J., & Layzer, J. B. (2010). Summer microhabitat use by adult and young-of-year snail darters (Percina tanasi) in two rivers. *Ecology of Freshwater Fish*, *19*(4), 609–617.

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science and Technology*, *48*(3), 1819–1827.

Bednarek, A. T., & Hart, D. D. (2005). Modifying dam operations to restore rivers: Ecological responses to Tennessee river dam mitigation. *Ecological Applications*, *15*(3), 997–1008.

Biggins, R., Eager, R., & Hurst, H. (1983). Snail darter recovery plan. Asheville, North Carolina: US Fish and Wildlife Service.

Boschung, H., & Mayden, R. L. (2004). *Fishes of Alabama*. Smithsonian Institution.

Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., & Miaud, C. (2011). Persistence of environmental DNA in freshwater ecosystems. *PLoS ONE*, *6*(8), 8–11.

Etnier, D. A. (1976). PERCINA (IMOSTOMA) TANASI, A NEW PERCID FISH FROM THE LITTLE TENNESSEE RIVER, TENNESSEE. *Proceedings of the Biological Society of Washington*, *44*(175), 469–488.

Etnier, D. A., & Starnes., W. C. (1993). *The Fishes of Tennessee. University of Tennessee Press,*

Knoxville. 681 p.

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, *4*(4), 423–425.

Ficetola, G. F., Pansu, J., Bonin, A., Coissac, E., Giguet-Covex, C., De Barba, M., … Taberlet, P. (2015). Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Molecular Ecology Resources*, *15*(3), 543–556.

Goldberg, C. S., Pilliod, D. S., Arkle, R. S., & Waits, L. P. (2011). Molecular detection of vertebrates in stream water: A demonstration using rocky mountain tailed frogs and Idaho giant salamanders. *PLoS ONE*, *6*(7).

Goldberg, C. S., Sepulveda, A., Ray, A., Baumgardt, J., & Waits, L. P. (2013). Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science*, *32*(3), 792–800.

Herzog, D. P., Ostendorf, D. E., Hrabik, R. A., & Barko, V. A. (2009). The mini-missouri trawl: A useful methodology for sampling small-bodied fishes in small and large river systems. *Journal of Freshwater Ecology*, *24*(1), 103–108.

Higgins, J. M., & Brock, W. G. (1999). Overview of Reservoir Release Improvements at 20 TVA Dams. *Journal of Energy Engineering*, *125*(1), 1–17.

Jane, S. F., Wilcox, T. M., Mckelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., … Whiteley, A. R. (2015). Distance, flow and PCR inhibition: EDNA dynamics in two headwater streams. *Molecular Ecology Resources*, *15*(1), 216–227.

Janosik, A. M., & Johnston, C. E. (2015). Environmental DNA as an effective tool for detection of imperiled fishes. *Environmental Biology of Fishes*, *98*(8), 1889–1893.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, *4*(2), 150–157.

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis stable, *47*(260), 583–621.

Neves, R. J., & Angermeier, P. L. (1990). Habitat alteration and its effects on native fishes in the upper Tennessee River system, east-central U.S.A. *Journal of Fish Biology*, *37*(sa), 45–52.

Pfleger, M. O., Rider, S. J., Johnston, C. E., & Janosik, A. M. (2016). Saving the doomed: Using eDNA to aid in detection of rare sturgeon for conservation (Acipenseridae). *Global Ecology and Conservation*, *8*(September), 99–107.

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, *51*(5), 1450–1459.

Service, U. S. F. and W. (2013). Snail Darter (Percina tanasi) Five-Year Review: Summary and Evaluation. *Federal Register*, (March 2013), 1–25.

Starnes, W. C. (1977). The ecology and life history of the endangered snail darter Percina (imostoma) Tanasi etnier. *Fisheries Research Report*, *77-52 ; Ii-144*.

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a Diverse Marine Fish Fauna Using Environmental DNA from

Seawater Samples. *PLoS ONE*, *7*(8), 1–9.

Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., … Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, *21*(11), 2565–2573.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3-new capabilities and interfaces. *Nucleic Acids Research*, *40*(15), 1–12.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., … Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char Salvelinus fontinalis. *Biological Conservation*, *194*, 209–216.

Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M., … Miya, M. (2017). Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Scientific Reports*, *7*(December 2016), 1–12.