Development of an Environmental DNA (eDNA) assay to Delineate the Distribution of the Imperiled Striated Darter (*Etheostoma striatulum*) in the Duck River, Tennessee

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BACKGROUND

The Striated Darter (Figure 2) is an exceedingly rare darter species whose distribution is limited to the Duck River watershed. Historically this species was known to occupy 16 tributaries to the Duck River. Surveys in 2006 (Abernathy and Mattingly 2011) found their distribution had been reduced to nine tributaries. However, the cryptic nature of this benthic fish makes it difficult to conclusively determine its present-day distribution. Due to their documented decline, Striated Darters are currently under review for federal listing under the Endangered Species Act. The comparison of our eDNA methods with traditional methods will enable the design of a robust and cost-effective protocol while also identifying critical habitat for the Striated Darter.

Molecular detection of discarded DNA fragments (eDNA) in environmental samples is a relatively new method for species detection and is well-suited for detecting species that are difficult to capture or have an elevated conservation status. A recent survey using a form of eDNA surveying (metabarcoding) successfully detected the Striated Darter in the main-stem Duck River, downstream of Shelbyville, TN.

Our objective is to develop an eDNA detection protocol (i.e., assay) to test for the presence of Striated Darters at all historically occupied sites and to delineate their current distribution within the Duck River.

METHODS

Assay Design and Optimization. We will be using a quantitative PCR (qPCR) approach for detecting Striated Darter DNA from filtered water samples. Primers and probes that are specific to Striated Darter DNA sequences are currently being optimized for an eDNA assay using the mitochondrial genes Cytochrome Oxidase Subunit (COI), Cytochrome B (CytB), NADH Dehydrogenase Subunit 2 (ND2), 16s rRNA, and 12s rRNA. Primers will be tested for specificity in *silico* using the Nucleotide Basic Local Alignment Search Tool (BLAST, Altschul et. 1990) and by using pairwise distance matrices built in MEGA-X (Kumar et al. 2018 v 10.1.8). Primer candidates will be tested for amplification efficiency and specificity *in vitro* using end-point PCR with tissue-derived DNA from non-target species (Figure 1).

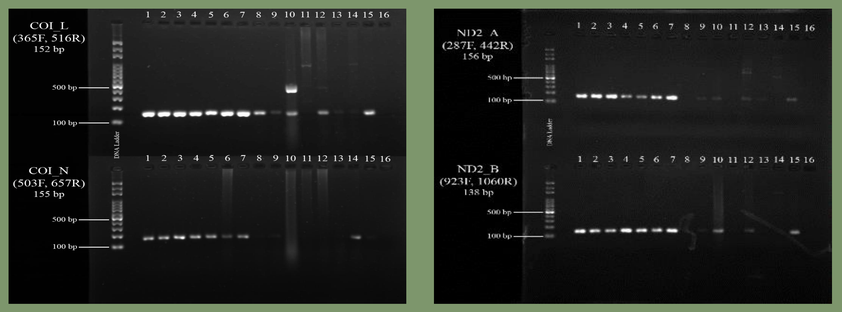

Figure 1. Selected primer assays under consideration for eDNA assays. Lanes 1-7 are individual Striated Darters from seven separate populations while lanes 8-15 are non-target species. Lane 16 represents the no DNA control. Bright bands indicate positive amplification while faded or empty bands indicate little to no amplification.

Figure 2. Striated Darter, *Etheostoma striatulum*, breeding male, 52mm standard length, North Flat Creek, Duck River, Tennessee. © Adam L. Walker

Figure 3a. Distribution of the Striated Darter in Tennessee represented by a filled in HUC-12 watershed. The Duck River is situated about 38 miles south of Nashville.

Field Sampling. A combination of two markers will be selected for our multiplex qPCR assay using optimized primers and probes to detect Striated Darter DNA from field samples. Water will be collected from a total of 30 historical sites throughout the Duck River drainage (Figure 3b.). Results from the qPCR reactions will be run through a hierarchical occupancy model (Positive = 1, Negative = 0) to test for the probability of detection at the qPCR replicate, sample replicate, and site level.

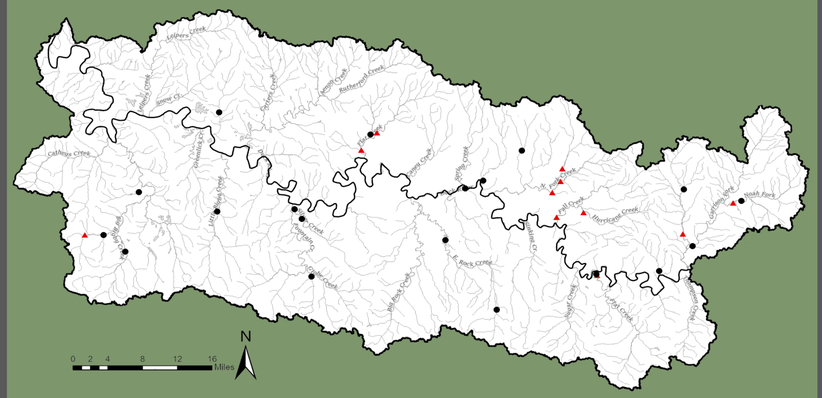

Figure 3b. Proposed eDNA sites (n = 30) to target Striated Darters within the Duck River system. Map represents the 12-digit hydrologic unit code (HUC-12) for their current distribution. Black circles represent sites where individuals were not captured in 2006 while red triangles represent sites where they were captured. Main-stem Duck River is noted by a bold, black line.

FUTURE METHODS AND DIRECTIONS

Each water sample will have three-to-five technical qPCR replicates using the optimized assay. Samples will be run alongside a dilution series of synthetic control DNA to enable quantification of DNA in unknown samples. If at least one qPCR replicate yields a positive amplification, the sample will be determined to have a positive detection. All positive qPCR reactions will be Sanger sequenced and compared to reference sequences to confirm that the correct target fragment was amplified (Figure 4).

After analysis of the eDNA site detection data, further work will be done to investigate their underlying population structure and overall genetic health. Genotyping by sequencing (GBS) will be used to generate a genome-wide dataset to identify and characterize single nucleotide polymorphisms (SNPs) across sampled populations of Striated Darters.

DISCUSSION

This assay will complement ongoing traditional sampling to identify critical habitat locations within the Duck River system. Positive detections through this eDNA assay will clearly define the Striated Darters distribution, allowing for focused management practices in the future.

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E_striatulum   CGGCGTAAAGCGTGGTTAAGATTATAATAAAACTAAAGCGGAATACCTCCAGAGCTGTTATACGCACC
E_smithi       CGGCGTAAAGCGTGGTTAAGATTATAATAAAACTAAAGCGGAATACCTCCAGAGCTGTTATACGCACC
E_virgatum     CGGCGTAAAGCGTGGTTAAGATGATAACAAAACTAAAGCCGAATACCTTCAAAGCTGTTATACGTGCC
E_blennioides  CGGCGTAAAGCGTGGTTAAGATTTTTCACAAAAATAAAGCCGAACACCTTCAGGGCTGTTATACGCACC
E_blennius     CGGCGTAAAGCGTGGTTAAGATTTTTACAAAAATAAAGCCGAACACCTTCAGGGCTGTTATACGCACC
E_caeruleum    CGGCGTAAAGCGTGGTTAAGGTTCCTTCAAAACTAAAGCCGAACACCTTCAGAACTGTTATACGCACC
E_crossopterum CGGCGTAAAGCGTGGTTAAGATTTAAACAAAACTAAAGCCGAACACCTTCAGAGCTGTTATACGCACC
E_cinereum     CGGCGTAAAGCGTGGTTAAGACTTACAAAAAACTAAAGCCGAACACCTTCAGAACTGTTATACGCACC
E_flabellare   CGGCGTAAAGCGTGGTTAAGACCATAACAAA-CTAAAGCCGAATACCTTCACAGCTGTTATACGCACC
E_flavum       CGGCGTAAAGCGTGGTTAAGATTTTTATGGAACTAAAGCCAAACACCTTCAGAGCTGTTATACGCACC
E_histrio      CGGCGTAAAGCGTGGTTAAGATTTTTATAAAAATAAAGCCGAACACCTTCAGGGCCGTTATACGCACC
E_kennicotti   CGGCGTAAAGCGTGGTTAAGATCATAACAAAACTAAAGCCAAATATCTCCAGAGCTGTTATACGCACC
E_luteovinctum CGGCGTAAAGCGTGGTTAAGATTATTTCAAAACTAAAGCCGAACACCTTCAGAACTGTTATACGCACC
E_nigripinne   CGGCGTAAAGCGTGGTTAAGATCATAACAAAACTAAAGCCAAATATCTCCAGAGCTGTTATACGCACC
E_obama        CGGCGTAAAGCGTGGTTAAGATTCTTACAATACTAAAGCCGAACACCTTCAGAGCTGTTATACGCACC
E_planasaxatile CGGCGTAAAGCGTGGTTAAGATTTTTACGAAACTAGAGCCGAACACCTTCAGAGCTGTTATACGCACC
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Figure 4. Sequence alignment file for a portion the mitochondrial 12S rRNA gene detailing base pair differences between *E. striatulum* and sympatric or closely related *Etheostoma* and *Nothonotus* species. Primers and probes will be designed for specificity to the Striated Darter.

REFERENCES

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ACKNOWLEDGMENTS