FY2022 R4 Inventory & Monitoring Branch Proposal Form

(no more than 4 pages)

Project Request

Title: Environmental DNA surveillance of mussel communities in the Hatchie River, Tennessee

Funding Requested ($)**:** $15,000

Project Type (Inventory, Monitoring, Research, Equipment Support): Monitoring and Research

Expected Project Completion Date: Expected Start Date: June 2023 and Expected End Date: June 2024

Submitted by and Contact Information: (Name, Title, Phone number, e-mail - of refuge staff member with direct project oversight responsibility)**:**

Amanda Rosenberger, U.S. Geological Survey, Assistant Unit Leader in the TN Cooperative Research Fishery Unit at Tennessee Tech University. arosenberger@tntech.edu; (931) 372-3239

Project Description

Primary Project Objective(s):

1) Use environmental DNA surveillance as an additional monitoring tool for determining the distribution of mussel species in the Hatchie River
2) Compare the accuracy and efficiency of eDNA monitoring to traditional monitoring techniques
3)

Briefly describe how this information will address refuge information needs (Link the needs to specific goals and objectives from the CCP, HMP, or a survey in the refuge Inventory and Monitoring Plan)**:**

Freshwater mussels represent a highly imperiled group of animals, with 70% of North American mussel species being classified as endangered, threatened, or of special concern (Neves et al. 1997). Habitat degradation, caused by factors such as land change, channelization, and impoundments, is attributed as one of the leading causes of declining mussel biodiversity. By studying mussels in their known habitats, researchers can quantify environmental factors conducive to mussel survivability and can provide specific feedback on how to improve instream conditions throughout historic ranges where mussels can no longer persist. Unfortunately, the true distribution of many mussel species is unknown, and distribution is a key piece of information needed to develop effective conservation measures.

Specifically, in West Tennessee, freshwater mussel records are scarce and thus the ability of resource managers to develop effective conservation strategies is limited. Ongoing and future restoration projects in the Hatchie River watershed would greatly benefit from updated mussel monitoring and inventories, but information is needed quickly. Environmental DNA (eDNA) surveillance

offers a fast and efficient tool to assist with traditional monitoring efforts for mussel surveillance. From the collection of small volume of water (e.g., 500mL), DNA within the water samples can be analyzed for the potential presence (or absence) of a species in the surrounding area.

A proposed project for the summer of 2023 has been submitted by Amanda Rosenberger and Kayla Key to survey and assess both mussel populations and habitats in the Hatchie River for the next three years. Through collaborative discussions, eDNA monitoring has been proposed as an additional surveillance tool that would greatly benefit the larger physical survey planned. Here, we propose an outline for a project that would work in conjunction with the planned 2023 mussel survey proposed by Rosenberger and Key. Our eDNA surveillance project would align with the goals of R4 refuges Inventory and Monitoring by providing validation of traditional surveillance data, and providing a new tool that can be used for years to come for quick and efficient mussel monitoring. Additionally, this project would add new information to the limited mussel records in West Tennessee. Pending results from this survey, additional funding would be applied for to continue surveillance within the Hatchie River refuges and beyond, for mussels, fishes, and other species of interests.

Briefly describe the sample design and methods (Include sample design and the number of sites, frequency of sampling and scale of effort; identify any protocols/SOPs that will be used (if cited, citation only))**:**

Ten sites will be sampled that correspond to areas within the Hatchie National Wildlife Refuge. Sampled sites will include sites that are surveyed with traditional monitoring to allow for comparison and validation between molecular and traditional surveillance.

Water samples will be collected using the Smith-Root eDNA sampler to filter water on site via peristaltic pumping. Water samples are typically collected near the surface. Previous eDNA research has shown that for benthic fish, samples taken from the surface compared to the benthic portion of the water column yield similar detection results (Paine et al. 2020;). However, samples will be collected near the benthic portion of the river if accessible. A total of 3-5 biological replicates will be collected over a 100-meter range at each site. Each biological replicate is comprised of a 2-liter water sample that has been filtered through 1.0µm pore-size, glass fiber filter. Filters will be aseptically placed in 5-mL storage tubes and preserved at -20 °C until further processing.

Samples are extracted by homogenizing a portion of the filter and purifying DNA using a DNA extraction kit formulated for water samples that have large amounts of inhibitors (e.g., wastewater, river water). Extracted DNA will be prepared for high-throughput sequencing using the Illumina '16S Metagenomic Sequencing Library Preparation' guide, and sequenced using the Illumina MiSeq Platform. This preparation guide and sequencing platform have been used for previous projects at Tennessee Tech and are easily modified to target various organisms (e.g., fish, mussels, bacteria). Two DNA assays will be used per Klymus et al. 2020. Two assays allow for better detection of multiple taxa and distinguishing between closely related taxa.

Filters will be stored at -80 °C for long term storage to be used for re-analyses, if necessary, or used for surveillance of other taxa, such as fish or aquatic insects.

Identify who will conduct the work (e.g., refuge staff-force account, contractor, or memorandum of agreement with federal/state/university/NGO partner):

Personnel from Tennessee Tech University and Tennessee West Basin Rivers Authority will work together to collect and preserve field samples. Dr. Robert T. R. Paine, is a Post-Doctoral Research

Assistant for the TN Coop Fish Unit and resident expert in eDNA surveillance. Dr. Paine will act as CO-PI and will be responsible for collecting field samples and all subsequent data generation and analyses. Dr. Amanda Rosenberger, as the PI, will have oversight over the data analyses and data interpretation.

Dr. Kayla Key of the Tennessee West Basin Rivers Authority is currently using eDNA surveillance techniques to collect water samples from Hatchie River tributaries and specializes in freshwater mussels. Dr. Key will provide logistical and field support.

Identify how the data be managed, and by who (Note – the Service should obtain copies of all data files as well as identify any data share agreements that preclude or restrict use of the information by either party)

The primary data generated from this project will be high-through sequencing data, which constitutes a large amount of data (10-20 GB). Given the large volume of data, physical copies are kept on-hand in the TN Coop Fish Unit in the form of data on external hard drives for data analyses. Data will also be stored in databases (GenBank, Sciencebase.gov) as a digital copy. Robert Paine will have primary oversight and management of data for analyses. Any additional files generated from analyses will also be stored on external hard drives and kept on-hand in the TN Coop Fish Unit and provided upon request and in full.

Identify project deliverables (e.g. map, data file, etc.) and time-frame in addition to a required Final Reports: All final reports will be based on a common template to be provided by the Inventory and Monitoring Branch.

- Map of surveyed area: June 2024
- List of detected species: June 2024
- Final Report: June 2024

Project Management

Identify the role of the requesting Complex or refuge(s) in this project:

We expect the role of the Complex or Refuges to be minimal, given that all sample collection, data generation, and data analyses can be completed by Tennessee Tech personnel.

Identify the principle investigator(s), affiliation, mailing address, email, and phone number if not refuge staff (e.g., partner led projects**):**

PI: Dr. Amanda Rosenberger, U.S. Geological Survey, Tennessee Tech University, TN Cooperative Fishery Research Unit, 1100 N. Dixie Ave, Box 5114, Cookeville, TN 38505. arosenberger@tntech.edu. (931) 372-3239

Co-PI: Dr. Robert T. R. Paine, Tennessee Tech University, TN Cooperative Fishery Research Unit, 1100 N. Dixie Ave, Box 5114, Cookeville, TN 38505. rtpaine@tntech.edu. 931-397-7207.

Identify any leveraging of cooperator or partner resources (e.g., In-Kind Services or Matching Funds**):**

Tennessee Tech University has a standard 42% indirect cost rate for all research funding proposals. However, the project funding mechanism for this proposal will be through a Research Work

Order, which falls under the Tripartite Cooperative agreement that guarantees a 15% indirect cost rate where research funding is given from state to federal agencies (i.e., R4 Refuges to U.S. Geological Survey). While no in-kind services or matching is required by R4 Refuges, we calculate the difference between the standard 42% indirect cost rate and 15% Tripartite Agreement indirect cost rate as in-kind support, which is calculated to be a 23% in-kind support.

Identify expected milestones and date of final deliverables:

Surveillance and sample collection would occur during the summer 2023, with sampling processing and analyses expected to take place during fall 2023. We expect to provide a final report on our findings no later than end of June 2024.

Identify the probable funding obligation mechanism – (Existing or New) (e.g., credit card, interagency agreement, sole-source contract, open source contract, Cooperative Ecological Studies Unit – CESU, other)

Project funding mechanism will be through a Research Work Order (RWO). A Research Work Order allows federal funds to be used by personnel in the Cooperative Research Fishery Unit for research and lower overhead cost.

Provide an itemized budget for requested funds (e.g., equipment, travel, labor, contract etc.):

Budget Category	Amount
Sample Collection	$85.00
DNA Extraction & Purification	$3,865.00
Metabarcoding Prep and Sequencing	$8,050.00
Travel	$1,500.00
Indirect Cost	$2,025.00
TOTAL	$15,525.00

Literature Cited

Klymus, K. E., Richter, C. A., Thompson, N., Hinck, J. E., & Jones, J. W. (2021). Metabarcoding assays for the detection of freshwater mussels (Unionida) with environmental DNA. Environmental DNA, 3(1), 231-247.

Neves, R.J., Bogan, A.E., Williams, J.D., Ahlstedt, S.A. and Hartfield, P.D. 1997. Status of aquatic mollusks in the southeastern United States: a downward spiral of diversity. pages 43-85. *in* Aquatic Fauna In Peril: The Southeastern Perspective G. W. Benz and D. E. Collins (editors), Special Publication, Southeast Aquatic Research Institute, Lenz Design and Communications, Decatur, Georgia.

Paine, R. T. R., Hurt, C. R., & Mattingly, H. T. (2021). Monitoring a minuscule madtom: Environmental DNA surveillance of the endangered pygmy madtom (*Noturus stanauli* Etnier & Jenkins 1980) in the Duck and Clinch rivers, Tennessee. *Environmental DNA*, 3(4), 745-759.

Proposal should be sent to: David_Richardson@fws.gov

Budget Justification

Travel: $1,500 – Requested funding will cover 6 overnight trips for 2 persons at the state rate ($96.00/night) totaling $1,200. The sampling schedule accounts for potential inclement weather based on previous experience during late spring and early summer where frequent precipitation events are normal. Sampling requires two people, and one hotel room will be provided for each person to adhere to current Covid-19 pandemic guidelines. Each person will be allotted per diem at the amount of $25.00 per day for each day totaling $300. Vehicles and boats will be provided by the Tennessee Tech Cooperative Fishery Research Unit, in which case, mileage is not allowed for federal vehicles.

Supplies: $12,000 – The supplies budget includes supplies that fit into three major categories: (1) Sample collection and water filtration, which includes supplies such as filters, storage tubes and disinfectant ($85.00), (2) DNA extraction, which includes supplies such as DNA extraction kits, pipette tips, and microcentrifuge tubes ($3,865.00), (3) Metabarcoding, which includes supplies such as sequencing reagents, pipette tips, microcentrifuge tubes, and DNA quantification kits for two assays ($8,050.00).

Indirect Costs: $2,025 – Indirect costs are calculated at 15% of total direct costs ($13,500) per the Tripartite Cooperative Agreement. Tennessee Tech's on-campus rate is 42% of total direct costs excluding capital expenditures (buildings, individual items of equipment, alterations and renovations), that portion of each subaward in excess of $25,000 and flow-through funds. The rate was set by the Department of Health and Human Services in an

agreement signed March 23, 2017, and effective through June 30, 2023. Tennessee Tech University defines a year as July 1 to June 30.

Research Work Order (RWO) Justification

The proposed project qualifies as a research work order under the guidelines that it helps support the professional development of a recently graduated, post-doctoral research associate. The project provides R.T.R. Paine with first-hand experience at designing, implementing, and coordinating a scientific project from the ground up; the supporting administration duties associated with a grant proposal; and the responsibilities in reporting to federal agency partners. The proposed project also helps increase partnerships between Paine and external agencies and provides the opportunity for manuscript publication.

This proposal and the information within has not yet been approved by the Office of Research at Tennessee Tech University. Information and calculations within are subject to change. In the event that alterations are needed, the PI and Co-Pi will inform and consult with the funding agency first.