Environment and Natural Resources Trust Fund
2019 Request for Proposals (RFP)

Project Title: **ENRTF ID: 165-D**

Genetic Analysis of Spiny Water Flea Invasion Sources

Category: D. Aquatic and Terrestrial Invasive Species

Sub-Category:

Total Project Budget: $ 337,942

Proposed Project Time Period for the Funding Requested: June 30, 2022 (3 yrs)

Summary:

This project uses genetic and genomic methods to determine the source water bodies from which spiny water fleas were carried to infest MN lakes.

Name: Michael McCartney

Sponsoring Organization: U of MN

Title: Research Assistant Professor

Department: CFANS, Fisheries, Wildlife and Conservation Biology

Address: 135 Skok Hall, 2003 Upper Buford Circle

St. Paul MN 55108

Telephone Number: (612) 301-7703

Email mmccartn@umn.edu

Web Address

Location

Region: Statewide, Northeast

County Name: Aitkin, Cook, Itasca, Lake, Lake of the Woods, Mille Lacs, St. Louis

City / Township:

Alternate Text for Visual:

Map of MN spiny water flea invasions

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%
____ If under $200,000, waive presentation?			

PROJECT TITLE: Genetic analysis of spiny water flea invasion sources

I. PROJECT STATEMENT This project applies genetic analysis techniques recently developed for zebra mussels to the study of source water for invasions of spiny water flea (SWF) in Minnesota (MN). Specifically, we will determine whether SWF in each MN lake originate from (1) Lake Superior, (2) other MN inland lakes, or (3) sources out of state. Also known as *Bythotrephes longimanus*, SWF is a species of crustacean zooplankton that inhabits deep, offshore waters of lakes. It grows to a length of < ½ inch and feeds carnivorously on native zooplankton. This puts it in direct competition with small fish (e.g., minnows, yellow perch, and cisco) that are the primary diet of gamefish. Invasions of SWF may lower growth rates and reduce populations of walleye and northern pike— two cornerstone species of Minnesota's multi-million-dollar angling industry.

Since 1990, when SWF was first detected in one of MN's inland lakes, the species has spread to about 35-40 more (see the included map), including some of the largest and most economically important basins in the state (e.g., Gunflint Lake, Lake Mille Lacs, Lake of the Woods, Lake Vermilion, Rainy Lake). The MN Department of Natural Resources, outreach and education agencies, and private citizen groups are aggressively seeking answers for how to prevent further spread of SWF. The next few decades represent a watershed moment in the state's efforts to prevent spread because if too many lakes become invaded, it becomes increasingly difficult to stop them.

- SWF may damage fish populations more than zebra mussels because SWF eat zooplankton
- SWF are spreading in MN but are still mostly restricted to northern lakes, so prevention has hope
- While intolerance of drying by adults and eggs suggests short distance spread, major "jumps" overland (e.g. to Lakes Mille Lacs and Vermillion) occur, with sources and vectors unknown
- Genotyping methods used have a proven track record in McCartney's work on zebra mussels
- Work on genome sequencing and SWF-specific genotyping methods is funded and underway
- This project nicely complements studies in the Branstrator lab on SWF ecology, tolerance to physical factors, and spread vectors associated with boating

II. PROJECT ACTIVITIES AND OUTCOMES **ENRTF BUDGET:** $126,306

Activity 1: Collection of SWF specimens from MN and sites out of state

Fifteen to 45 specimens per lake will be collected from all infested MN inland lakes and from putative source waters in nearby states and Canada, including the following: Gile Flowage (northern WI, 2003 first detection), Lake Mendota, (near Madison, WI, 2009), Lake Michigamme (UP Michigan, 1994), Lake Michigan (1986), Lake Superior (1987), Lake Nipigon, Ontario (2001), Lake Winnipeg, Manitoba (2010), and Stormy Lake (northern WI, 2007). About half of the MN inland lakes have no drivable water access. Great Lakes will be sampled from multiple sites. Within-lake sampling in lakes where SWF are abundant will be used to examine the diversity of clones (SWF females reproduce by asexual eggs in the summer). All samples will be preserved in ethanol.

Outcome	Completion Date
1. Sampling from MN	September 2019
2. Sampling out of state and in Canada	September 2020

Activity 2: Genotyping to determine invasion source waters **ENRTF BUDGET:** $211,637

SWF adults are tiny (each 1/10,000th of an ounce), but we found that DNA extraction followed by amplification of the whole genome produces high yield of high quality DNA. Each amplified genome will be processed using Sequence Based Genotyping at the U of M Genomics Center (UMGC) using methods optimized for the SWF genome in our pilot study. Single Nucleotide Polymorphism (SNP) data will be computer-processed and filtered in the McCartney lab to remove missing and noisy data. Filtered SNP genotypes will be placed into genetic clusters

using Admixture software—clusters provide evidence for the sources of MN lake invasions. Independent and statistically rigorous contrasts of invasion scenarios (1 = Lake Superior origins; 2 = origins from other MN inland lakes; 3 = origins out of state) will be done using Approximate Bayesian Computation (ABC) modeling. Lakes can be infested by both asexual and sexual offspring of SWF (the latter derive from "resting eggs" that overwinter and are more tolerant of physical stressors). To test the hypothesis of limited dispersal of the asexual life phase, we will examine the geographic distance over which clones are distributed across lakes, and examine one lake in detail throughout the year to determine how diversity of clones changes seasonally. Laboratory-raised broods hatched from asexual eggs will be genotyped to check the expectation that they have identical genotypes.

Outcome	Completion Date
1. Sequence Based Genotyping data collection	July 2021
2. Analysis of data, invasion model testing to determine pathways	December 2021
3. Manuscript preparation	June 2022

III. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role
Michael A. McCartney	Research Assistant Professor	UM Twin Cities	Project director, sample collection, data analysis, manuscripts
Donn K. Branstrator	Associate Professor	UM Duluth	Sample collection, live animal work, manuscripts
Sophie Mallez	Postdoctoral Research Assoc.	UM Twin Cities	Sample collection, lab work, data analysis, manuscripts

IV. LONG-TERM- IMPLEMENTATION AND FUNDING:

Invasion genetic analysis provides direct evidence bearing on invasion pathways— "invasion forensics," as it has been called. This is crucial for SWF in this early phase of geographic range expansion in MN, as we have much less information on spread than we have for other aquatic invasive species, and essentially no options for control at this time. Finding chronic introductions from nearby lakes would reinforce the need to prevent short-distance spread by recreational water-users to identify the activities that spread SWF. Repeated introductions from Lake Superior would identify a whole other category of recreationalists, and introductions from out of state another still. This information would be used to better narrow the focus of prevention education and outreach in the geographic region in which SWF is currently spreading. We will disseminate our research findings and outreach message to a broad list of stakeholders which includes lake associations, anglers, boaters, recreational users and outfitters on canoe wilderness areas, agency personnel, researchers, and policy makers. Presentations to MN Department of Natural Resources staff will be made to share and interpret findings as they relate to spread prevention investments and strategies for SWF. Presentations will be made to lake associations in infested and uninfested regions of MN to build awareness, education, and civic engagement.

V. TIME LINE REQUIREMENTS:

We anticipate one full year required to complete collections, two years in total to genotype samples, 2.5 years to complete analysis and 3 years total from start to completion of the project, including the completion of at least two manuscript drafts resulting from the work.

2019 Proposal Budget Spreadsheet

Project Title: Genetic analysis of spiny water flea invasion sources

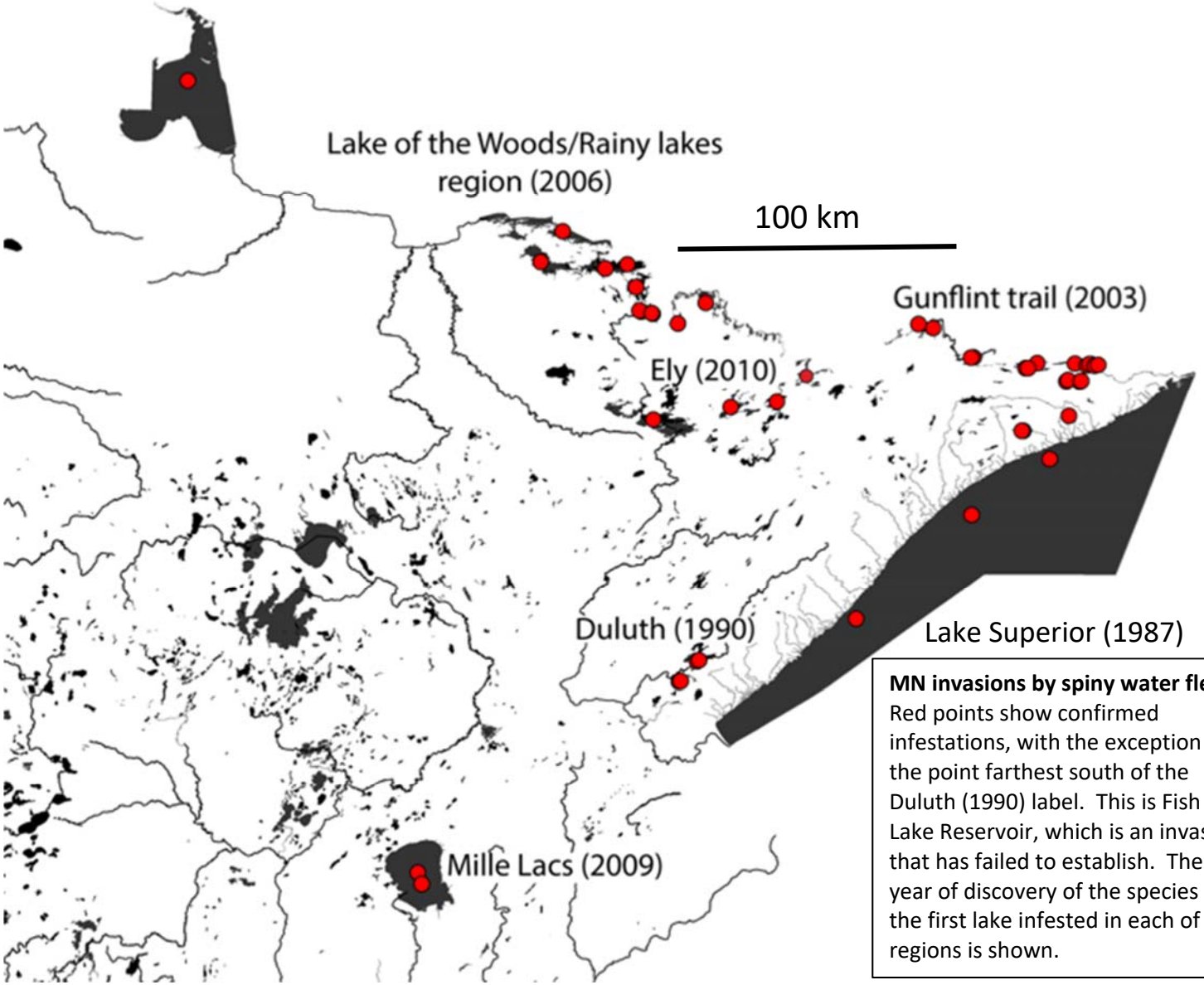
IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM (See "Guidance on Allowable Expenses")	**AMOUNT**
Personnel: 1. Michael McCartney, Research Assistant Professor, 25% FTE per year, 74.8% salary, 25.2% fringe benefits, 3 years. 2. Donn Branstrator, Associate Professor UM Duluth, 16.6% FTE per year, 74.9% salary, 25.1% fringe benefits, 2 years. 3. Sophie Mallez, postdoctoral associate, 50 % FTE per year, 81.7% salary, 18.3% fringe benefits, 3 years.	$ 278,912
Professional/Technical/Service Contracts: Fees for Sequence Based genotyping of spiny water fleas to be paid to the UM Genomics Center. Estimated 1,665 specimens at $16.07 processing fee per specimen + $1,125 for 1 run on Illumina iSeq	$ 27,884
Equipment/Tools/Supplies: 1. Field supplies (tow nets, ethanol, collection vials). 2. Laboratory supplies including DNA extraction kits, whole genome amplification kits, ethanol and plasticware for preparing samples prior to genotyping.	$ 15,390
Travel: Vehicle mileage, hotel and meals = $5,212. Approximately half of this total is domestic travel to collecting sites in WI and MI. Without analyzing these samples, we cannot confidently determine the sources of the MN infestations, and the collections are too numerous to rely upon collectors. Collections in Canada (2 lakes only) are being mailed to avoid international travel needs. Conference attendance is 3 in-state AIS meetings to accomplish outreach.	$ 8,212
Additional Budget Items: 1. Boat rental: small boat for collecting from inland lakes = $2,424 2. Rental of Kingfisher from UMD Large Lakes Observatory fleet for Great Lake collecting = $4,120	$ 6,544
Shipping costs to send water flea samples from UMD to UMN, and shipping from collectors in Canada.	$ 1,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 337,942

V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ To Be Applied To Project During Project Period:	$ -	
Other State $ To Be Applied To Project During Project Period: University of Minnesota, Duluth Chancellor's Faculty Small grant awarded to D. Branstrator for sequencing of SWF genome and pilot study to develop genotyping methods	$2,635	Secured
In-kind Services To Be Applied To Project During Project Period:	$ -	
Past and Current ENRTF Appropriation:	$ -	
Other Funding History:	$ -	

B. Visual Component or Map



MN invasions by spiny water flea. Red points show confirmed infestations, with the exception of the point farthest south of the Duluth (1990) label. This is Fish Lake Reservoir, which is an invasion that has failed to establish. The year of discovery of the species in the first lake infested in each of 6 regions is shown.

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D. Acquisition, Easements, and Restoration Requirements
This is not an acquisition or restoration proposal

F. Project Manager Qualifications and Organization Description
At the University of Minnesota, Twin Cities
Michael McCartney is a Research Assistant Professor in the Minnesota Aquatic Invasive Species Research Center (MAISRC) and the Department of Fisheries, Wildlife and Conservation Biology at the University of Minnesota, Twin Cities. He holds a Ph.D. in Ecology and Evolution from the State University of New York (1994), has 5 years of post-doctoral experience at the Smithsonian Tropical Research Institute, University of California and Florida State University, and spent 13 ½ years on the faculty at the University of North Carolina, Wilmington, studying marine and freshwater aquatic animals. He has more than 20 years of research experience on using genetic techniques to study the geography of native and invasive populations of aquatic organisms, and has published his work in the journals *Evolution, Ecology, PLOSOne, Journal of Biogeography, Biological Invasions* and *Aquatic Invasions,* among others.

McCartney leads the invasive mussel research program at MAISRC where he focuses on zebra mussel ecology, population genetics and genomics for the purpose of understanding and preventing spread, and he maintains active research aimed at developing population controls. He will direct and assist with research on genotyping spiny water flea. The recently published work by Drs. Mallez and McCartney on genetics of zebra mussel invasion sources provides the methods and approach upon which this proposal was modeled.

ORGANIZATION
The flagship University of Minnesota, Twin Cities is the state's land-grant university and one of the most prestigious public research universities in the nation.

At the University of Minnesota, Duluth
Donn K. Branstrator is an Associate Professor of Biology at the University of Minnesota Duluth. He obtained the PhD from the University of Michigan (Ann Arbor, Michigan) and continued postdoctoral study at the University of Montreal and McGill University (Montreal, Canada). He has published a variety of papers in the primary literature on zooplankton ecology including 8 papers in *Limnology & Oceanography*, served as primary advisor of 15 graduate students (12 MS, 3 PhD), currently sits on the Swenson College of Science and Engineering Executive Committee, currently sits on the Mille Lacs Fisheries Advisory Committee, and was recently the Director of Graduate Studies for the Integrated Biosciences Graduate Program at the University of Minnesota. His research focuses on the ecology of invasive zooplankton with emphasis on spiny water flea. He teaches undergraduate and graduate lecture and laboratory courses in general ecology, lake ecology, aquatic food webs, and invasion biology.

ORGANIZATION
The University of Minnesota Duluth is a branch of the University of Minnesota, a public institution with missions in teaching, research, and service.