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

Project Title: **ENRTF ID: 060-B**

Dangers of Nanoparticles on Aquatic Health

Category: B. Water Resources

Sub-Category:

Total Project Budget: $ 494,075

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

Summary:

We will determine the dangers of nanoparticles to aquatic bacteria and fish health, enabling us to provide recommendations about safe levels and to predict how new nanomaterials might affect aquatic species.

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Sponsoring Organization: U of MN

Title:

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Study the dangers of nanoparticle exposure to fish and microbes in our waterways. Work will be completed by Erin Carlson at UMN – Twin Cities Minneapolis, MN Chemical Biology; Jeff Gralnick at UMN – Twin Cities St. Paul, MN Microbiology; Jennifer Liang at UMN – Duluth Duluth, MN Zebrafish Biology

_____ 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: Dangers of Nanoparticles on Aquatic Health

I. PROJECT STATEMENT

Nanoparticles are tiny objects with at least one dimension less than 100 nanometers – 10,000 or more nanoparticles can fit on the head of a pin. Nanoparticles have many valuable properties, which is why their use has become widespread in a variety of everyday products, ranging from sunscreens and antimicrobial clothing to batteries and computer displays. **The increasing usage of these materials and their inevitable release into our environment has created a need for information about how nanoparticles will affect our culturally important and economically valuable rivers and lakes.** Nanoparticles are thought to be toxic because their small size allows them to be inadvertently consumed by all forms of wildlife, exposing them to high levels of the metals that make up these tiny particles. Thus, the increased usage of nanoparticles has created a potential danger to aquatic organisms. Nanoparticles find their way into aquatic environments as their small size makes them very hard to contain. The Minnesota Pollution Control Agency has been assessing the increasing abundance of nanoparticles in our waterways since 2009. **A greater understanding of the dangers of nanoparticle exposure to aquatic species is needed to make recommendations about what environmental levels may be considered safe and to better predict the safety of new materials as they are introduced.** This proposal will address this need by identifying impacts of nanoparticles on the health of aquatic organisms and creating an accurate, efficient way to measure these health impacts. This proposal responds specifically to Priority B.2.I: Understanding the impacts of contaminants on the health of…aquatic species.

All higher organisms, from fish and frogs to humans have diverse communities of micro-organisms within their bodies, the "microbiome", that play an essential role in health and disease. Although nanoparticles are already abundant in our lakes, rivers, and streams, the dangers they pose to fish health are poorly understood. Our hypothesis is that nanoparticles will cause changes in the fish microbiome that will adversely affect their health. **The proposed work will develop an easily manipulated and controlled fish-microbiome system that will enable us to define how nanoparticles affect the fish microbiome and fish health.** Zebrafish, a small freshwater fish, will be used as the fish system. Zebrafish can be raised in the laboratory without a microbiome. Specific bacteria can then be added to the microbiome-free fish to create a rigorous experimental system. For the bacteria, this project will initially use *Shewanella,* a species commonly found living in the intestinal track of zebrafish and in fish from Minnesota lakes and rivers. Thus, the zebrafish-*Shewanella* system represents an excellent model system to explore how nanoparticles influence animal and microbiome health. In preliminary studies, we found that *Shewanella* becomes "resistant" following exposure to a nanoparticle commonly used in batteries (nickel manganese cobalt oxide). This striking result indicates that nanoparticles are likely to have dramatic effects on most bacteria, as well as the microbiomes of many other aquatic organisms. Our research will span several scales, from working with isolated bacteria, to fish colonized with a single bacterial species, to fish that have their natural microbiome. **The critical outcome of this work is a better understanding of the dangers that nanoparticles pose to aquatic species, and the foundational knowledge required to develop appropriate regulations for these new materials.**

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: *Define effects of industry-generated nanoparticles on aquatic bacteria.*
Description: The first step will be to define the effects of nanoparticle on *Shewanella* bacteria isolated from live zebrafish. Focus will be placed on nanoparticles that are currently of greatest concern to the EPA: nanosilver (clothing), quantum dots (electronic displays), and metal oxides (cosmetics, sunscreen, batteries). These experiments will include direct assessments, such as measuring survival, growth, and reproduction of the bacteria, to experiments that use cutting edge techniques – genomics, proteomics and metabolomics – that will comprehensively characterize the response of *Shewanella* to nanoparticles. These experiments on the simplest system, isolated bacteria, will enable us to build specific hypotheses about how different

nanoparticles will affect the more complex fish-microbiome system (Activities 2 and 3).

ENRTF BUDGET: $ 172,896

Outcome	Completion Date
1. Develop workflow for examination of bacteria in the presence of multiple nanoparticle types	Dec 31, 2019
2. Biochemical assessment of multiple bacterial strains following particle exposure	June 1, 2021
3. Generate and validate hypotheses about how microbiome health will be affected	June 1, 2022

Activity 2: *Evaluate the effects of industry-generated nanoparticles on simple and complex microbiomes in the gut of live zebrafish.*

Description: The next step will be to determine how nanoparticles affect the microbiomes within live zebrafish. The gut of zebrafish raised without a microbiome will be populated with pure *Shewanella* or with a more normal, complex complement of microorganisms. The changes in the microorganisms in response to nanoparticles will be measured using the same set of assays as in Activity 1. The priority will be to test the most reactive nanoparticles from Activity 1.

ENRTF BUDGET: $ 181,629

Outcome	Completion Date
1. Meta-omic analysis of system prior to nanoparticle exposure	June 1, 2020
2. Meta-omic analysis of system in response to nanoparticles	June 1, 2021
3. Test Shewanella *mutants lacking highly expressed genes from nanoparticle exposure*	June 1, 2022

Activity 3: *Characterize the effects of nanoparticles on the development and health of zebrafish colonized with simple and complex microbiomes.*

Description: Determine how development and health of larval fish is altered when nanoparticles are added to germ-free fish, fish carrying only *Shewanella,* and fish carrying a normal, complex complement of microorganisms. Assays will include direct measures of health such as growth, development, and function of organ systems, as well global analysis of changes in gene expression through transcriptome analysis. These studies will enable us to identify highly vulnerable tissues of aquatic vertebrates.

ENRTF BUDGET: $ 139,550

Outcome	Completion Date
1. Define how fish development is affected by the presence of nanoparticles	June 1, 2020
2. Analyze the effects of nanoparticles on fish health and growth	June 1, 2021
3. Identify genes affected by nanoparticles through transcriptome analysis	June 1, 2022

III. PROJECT PARTNERS:

A. Partners receiving ENRTF funding: N/A

B. Partners NOT receiving ENRTF funding: N/A

IV. LONG-TERM- IMPLEMENTATION AND FUNDING:

At the end of the three-year project, we expect to have obtained results that will support funding from national agencies (e.g., National Science Foundation, Department of Energy, National Institutes of Health) and we will begin partnering with organizations (such as Natural Resources Research Institute) and local companies to provide guidance on the regulation of nanoparticle release into Minnesota waterways.

V. TIME LINE REQUIREMENTS:

Three years of funding will be sufficient to accomplish the examination of how organisms respond to nanoparticle exposure in increasingly complex systems: pure bacterial cultures (Activity 1), simple and complex microbiomes in the gut of live zebrafish (Activity 2) and the development and health of colonized zebrafish (Activity 3).

2019 Proposal Budget Spreadsheet

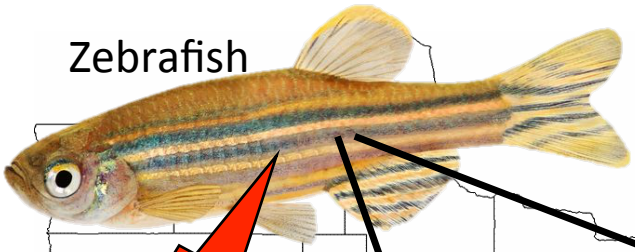
Project Title: Dangers of Nanoparticles on Aquatic Health

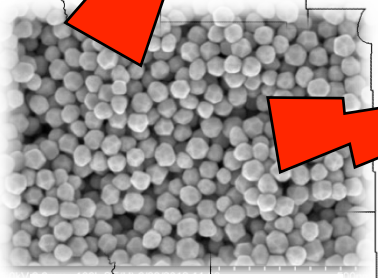
IV. TOTAL ENRTF REQUEST BUDGET *3* **years**

BUDGET ITEM (See "Guidance on Allowable Expenses")	**AMOUNT**
Personnel: Carlson (PI) 4% for 3 yrs; advise one graduate student on chemistry-related experiments and on interpreting changes in microbial abundance and biochemical processes related to nanoparticle treatments; 75% salary, 25% benefits, $25,677. Gralnick (co-PI) 6% for 3 yrs; advise one graduate student on microbiology-related experiments and on interpreting changes in microbial abundance and physiology related to nanoparticle treatments; 75% salary, 25% benefits, $26,754. Liang (co-PI) 8% for 3 yrs; participate and guide the experiments in Aim 3 of this proposal. She will train and mentor the graduate student, Senior Laboratory Technician, and undergraduate students participating in this project; 75% salary, 25% benefits, $38,448. Advanced Grad. Student (1) 75% for 3 yrs; lead experiments in Activity 1 and participate in Activity 2; 80% salary, 20% benefits, $74,719. Beginning Grad. Student years 2 and 3 (1) 50%; lead experiments in Activity 2; 65% salary, 35% benefits, $90,374. Beginning Grad. Student (1) 100% for year 3; lead the majority of experiments in Activity 3; 54% salary, 46% benefits, $41,912. Senior Laboratory Technician (1) 20% for 3 years; train students in new techniques for Activity 3. Importantly, this technician will provide help in problem solving, including ensuring continuity and consistency of techniques and experimental design; 75% salary, 25% benefits, $26,114. Undergraduate (1) for 3 years; independent projects that focus on analyzing a specific developmental or health change in the nanoparticle exposed larva; 100% salary, $3,076.	$ 327,075
Professional/Technical/Service Contracts: Mass spectrometry service contact (Carlson Lab), required to maintain and repair instrumentation - Agilent Technologies for Q-TOF	$ 22,500
Equipment/Tools/Supplies: (Carlson): chemicals, tubes, plates, media components, nanoparticles, mass spectrometry supplies and laboratory supplies ($19,000). (Gralnick): chemicals, molecular biology reagents and laboratory supplies. We also are requesting $3,000 / year for maintenance and management of zebrafish tanks in the College of Biological Sciences Teaching Lab Facility that will be used for the proposed experiments ($27,000). (Liang): consumable plastic and glass supplies such as microfuge tubes, slides, Petri dishes, and supplies for maintenance of the fish facility and fish, such as food and filters. They also include biochemical and chemical reagents required for the analysis of gene expression and developmental time points ($22,000).	$ 68,000
Acquisition (Fee Title or Permanent Easements):	N/A
Travel: Funds for research groups to travel for All-Hands meeting once a year (to Duluth or Twin	$ 1,500
Additional Budget Items: UMN Lab facilities/services such as proteomics facility, NMR, confocal laser scanning microscope, DNA sequencing, RNA-Seq analysis.	$ 75,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 494,075

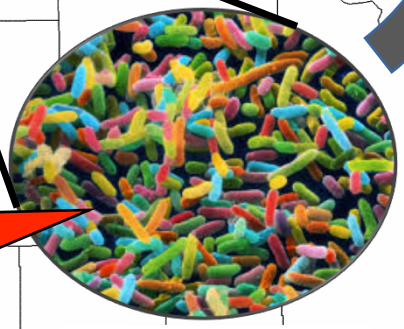
V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ To Be Applied To Project During Project Period:	N/A	
Other State $ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period:	N/A	
Past and Current ENRTF Appropriation: Co-PI Gralnick is also a Co-PI on the ENRTF award "Reducing Salt and Metal Removal Costs with Microbes" (046-B, PI - Daniel Bond) awarded to UMN. In total, he has ~1 year of funding left on this project (~ $200,000) which is scheduled to expire in June 2019. **This project is unrelated to the proposed work.**	~$200,000	Expires June, 2019
Other Funding History:	N/A	



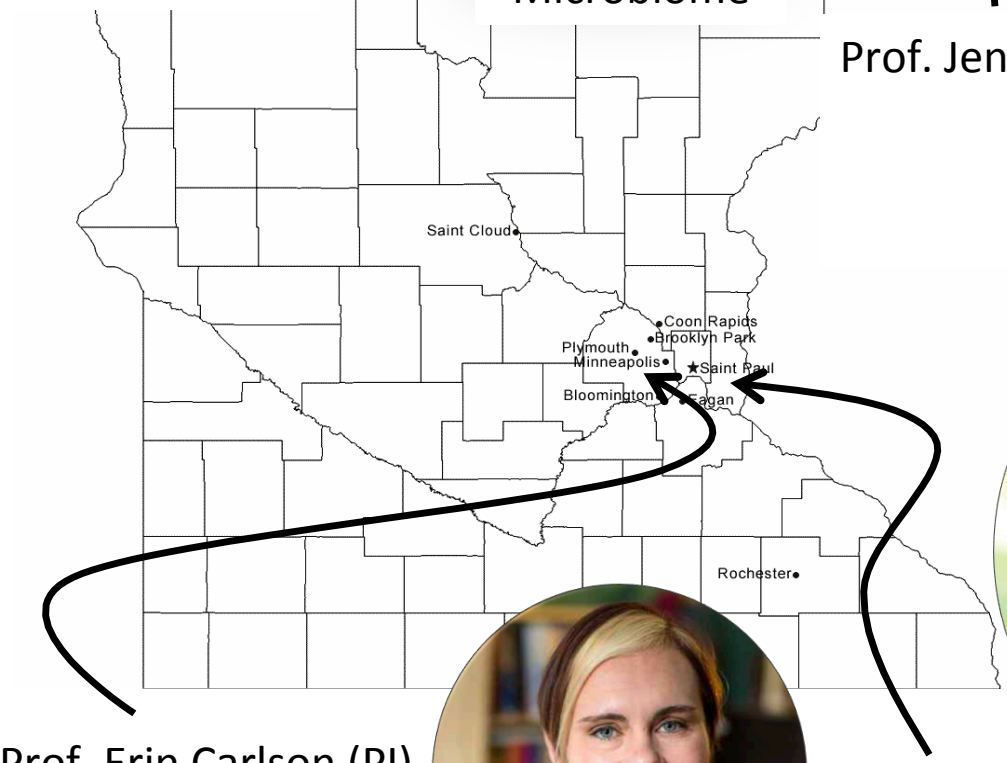


Nanoparticles



Fish Microbiome

Prof. Jennifer Liang (Co-PI)
UMN – Duluth
Duluth, MN
Zebrafish Biology



Prof. Jeff Gralnick (Co-PI)
UMN – Twin Cities
St. Paul
Microbiology

Prof. Erin Carlson (PI)
UMN – Twin Cities
Minneapolis
Chemical Biology

VI F. Project manager qualifications & organization description

Project Manager Qualifications

Dr. Erin E. Carlson, Project Manager and Principle Investigator

Dr. Carlson is an Associate Professor of Chemistry at the University of Minnesota. She is also affiliated with the departments of Biochemistry, Molecular Biology, and Biophysics and Medicinal Chemistry. Carlson was trained in Chemistry at the University of Wisconsin – Madison where she specialized in organic synthesis, enzymology, and microbiology. She then spent 2.5 years at The Scripps Research Institute (La Jolla, CA) where she developed novel mass spectrometry-based methods for metabolite analysis. Her training in chemistry, -omics analysis and microbiology will provide cohesiveness for our interdisciplinary team. Importantly, she also has extensive expertise in the area of nanoparticle toxicity assessment and is currently a member of a large interdisciplinary team (Center for Sustainable Nanotechnology funded by the National Science Foundation) devoted to investigating the molecular mechanisms that dictate nanoparticle interactions with biological systems. Please visit her website for further description of research interests: http://www1.chem.umn.edu/groups/carlson/.

Dr. Carlson is the Project Manager of this proposal. Since the start of her independent career, she has a strong record of obtaining funding and she has been recognized with numerous awards including being named a Presidential Early Career Awards for Scientists and Engineers (PECASE) recipient, a Pew Biomedical Scholar, the NIH Director's New Innovator Award, the Indiana University Outstanding Junior Faculty Award, the NSF CAREER Award, the Cottrell Scholar Award and was named a Sloan Research Fellow, an Indiana University Dean's Fellow and an ACS WCC Rising Star. Dr. Carlson was also named Outstanding Postdoctoral Mentor of 2017 by the University of Minnesota Postdoctoral Association.

Organizational Description

The University of Minnesota is proud of its history as a land grant research university and is dedicated to its mission of promoting access to higher education and collaborating to advance knowledge benefiting communities, the state, and world. The University of Minnesota's Mission is threefold: 1) Research and Discovery, 2) Teaching and Learning, and 3) Outreach and Public Service.