

**Environment and Natural Resources Trust Fund  
2016 Request for Proposals (RFP)**

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**Project Title:**

**ENRTF ID: 052-B**

Perfluorochemical Contamination Effects on Amphibians and Wetland Ecosystems

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**Category:** B. Water Resources

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**Total Project Budget:** \$ 250,954

**Proposed Project Time Period for the Funding Requested:** 2.5 years, July 2016 to December 2018

**Summary:**

Chemical contamination puts aquatic ecosystems at risk. We will measure perfluorochemical contamination in wetlands and effects on frog survival/ development to identify risks to declining amphibian populations and wetland ecosystems.

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**Location**

**Region:** Statewide

**County Name:** Statewide

**City / Township:**

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**Alternate Text for Visual:**

Artificial pond experiment will test effect perfluorochemicals (PFCs) and interactions with environmental stressors (nitrates and accelerated drying) on tadpole development in native frogs. Perfluorochemical concentrations will be quantified in isolated depressional wetlands (vernal pools, prairie wetlands) from forested and prairie regions of Minnesota.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____ %



**PROJECT TITLE: Perfluorochemical contamination effects on amphibians and wetland ecosystems**

**I. PROJECT STATEMENT**

Chemical contamination is a major concern for aquatic ecosystems across Minnesota. Perfluorochemicals (PFCs), including PFOA and PFOS, have been associated with considerable environmental risk due to their ubiquitous nature, persistence, and potential to impact wildlife. PFCs have been used in industrial, commercial, and military products and released to the environment through manufacturing, chemical spills, breakdown of surfactants and fire-fighting foams, and industrial/personal use. PFCs have now been detected across the globe in drinking water, lakes, soils, wastewater effluent, humans and wildlife; this widespread contamination is of great concern because many PFCs are resistant to degradation, toxic to animals, and have the ability to bioaccumulate.

Minnesota has been on the forefront of environmental regulation of these emerging contaminants. However, little is known about toxicity of these chemicals in nature and their seasonal peaks in ephemeral aquatic habitats, which only hold water for a few months after snowmelt. Ephemeral vernal pools and prairie wetlands are widespread in Minnesota and of critical importance to a variety of organisms, particularly frogs. These wetlands have high potential for increased concentrations of PFCs due to their natural drying cycle, further worsened by the climate change. In addition, these habitats have additional stressors such as excess nutrients from nearby agricultural lands. Amphibian survival and health – an excellent indicator of the state of the entire aquatic ecosystem - could be compromised due to direct effects of PFCs on frog survival and development as well as disruption of behavior and effects on the food base. This information cannot be deduced from single-species toxicology assessments done to date, yet it is essential for improved understanding of PFC toxicity in the natural setting and its effects on the entire food web.

Our goal is to determine toxicity and sub-lethal effects on frogs and assess PFCs in drying wetlands. We will determine concentrations of this persistent pollutant in a representative subset of ephemeral MN wetlands and test the toxicity of PFCs with native frogs. This study will include additional stressors that could impact frog survival, growth, and development, including water level fluctuations (these systems are currently threatened by increased water loss due to climate change), and nitrate concentrations (often elevated in these systems and known to increase mortalities and abnormalities in frogs). This proposed work is novel because previous research does not evaluate direct and indirect effects of environmentally relevant concentrations in native frog species with additional stressors that are likely to occur. The results from this project will provide needed information to: a) identify risks to wetlands with high ecological value; b) understand the impacts of contaminants on the health of aquatic species; c) advance development of standards for contaminants; and d) understand how to mitigate climate change impacts. This project will benefit from the existing artificial pond (mesocosm) system for ecosystem-level ecotoxicological studies with native frog species, and NRRI efforts to map and characterize land use done for two federally funded projects.

**II. PROJECT ACTIVITIES AND OUTCOMES**

**Activity 1:** Determine toxicity of PFCs for tadpole survival, growth, and development **Budget: \$125,206**

Tadpoles will be exposed to real-life levels of PFCs in an outdoor small pond experiment to assess dose-response effect on survival, size, development, health, and gonadal development. Experiments will include environmentally-relevant PFC levels, including 0 (none detectable), the MN established health risk level in drinking water (0.3 µg/L), and levels found at spills (40-4,000 µg/L). Furthermore, we will determine how toxicity may be increased in the presence of water level changes and nitrate concentrations.

Outcome	Completion Date
1. Data on PFS concentrations causing direct and indirect effects of PFCs on frog survival, growth, development, and abnormalities	Aug – Dec 2017

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2. Quantitative data on the synergistic effects of PFCs and pond drying and nitrates	Aug – Dec 2017
3. Updated guidelines for a range of toxicity guidelines	Feb 2018

**Activity 2:** Determine levels of PFCs in ephemeral wetlands across Minnesota, identify land use practices that increase exposure **Budget: \$120,870**

Vernal pools and prairie wetlands may accumulate higher levels of PFCs than the surrounding landscape, resulting in levels of exposure exceeding state-recommended guidelines. We will determine levels of these compounds in a representative sample of Minnesota ephemeral wetlands, sampled closer to the end of pond-drying cycle (Mid-June-early July).

Outcome	Completion Date
1. Determine end-of season PFCs concentrations in 20 vernal pools and 20 prairie wetlands	Aug 2018
2. Identify land management practices associated with greater concentrations, develop guidelines for natural resource management	Dec 2018

**Activity 3:** Outreach on direct and indirect effects of PFCs and risks to amphibians **Budget: \$4,878**

Outcome	Completion Date
1. Disseminate information to state resource management agencies (MNDNR, MNSG etc)	Aug 2018
2. Conduct 2 workshops per year for the general public on risks to amphibian populations in MN, including legacy pollution and contaminants of emerging concern	Aug 2018
3. Educational materials (printed and digital) on risks to amphibian populations in MN	Dec 2018

**III. PROJECT STRATEGY****A. Project Team/Partners**

Dr. Jennifer Olker (PI), Dr. Katya Kovalenko (co-PI) and Dr. Igor Kolomitsyn (organic chemist). Dr. Olker and Kovalenko will direct activities 1-3, Dr. Kolomitsyn will provide expertise in organic chemistry and PFCS quantification. All from University of Minnesota Duluth NRRI.

**B. Project Impact and Long-Term Strategy**

Our new approach considering combined effects of PFCs and other stressors will improve natural resource conservation in Minnesota by providing information for long-term exposure toxicity guidelines and risk assessments. This information will be useful for agencies that regulate and/or monitor these compounds, e.g. MNSG, MN-DNR, MPCA. It is essential to obtain these data to support and improve current PFC standards implemented by the state, enhance risk management decisions and select most appropriate remediation strategies. This project is part of a larger research effort led by Dr. Olker to understand and mitigate the ever-accelerating declines in amphibian populations, and it also targets to address concerns about water quality in aquatic ecosystems across Minnesota highlighted in the recent MPCA report.

**C. Timeline Requirement**

Two years; start date needs to accommodate natural amphibian breeding dates to obtain eggs for the mesocosm experiment (May).

## 2016 Detailed Project Budget

**Project Title: Perfluorochemical contamination effects on amphibians and wetland ecosystems**

<b>IV. TOTAL ENRTF REQUEST BUDGET 2 years</b>	
<b>BUDGET ITEM</b>	<b>AMOUNT</b>
<b>Personnel:</b>	
Jennifer Olker, Project Manager (66.3% salary, 33.7% benefits); 25% FTE each year for 2 years	\$ 36,785
Katya Kovalenko, Co-Investigator (66.3% salary, 33.7% benefits); 25% FTE each year for 2 years	\$ 40,988
Igor Kolomitsyn, Organic Chemist (66.3% salary, 33.7% benefits); 25% FTE each year for 2 years	\$ 45,452
Graduate Research Assistant, manage mesocosm exposure and field collections (59% salary, 42% benefits and tuition reimbursement); 50% FTE each year for 2 years	\$ 89,980
Field Technician, assist graduate research assistant with mesocosm and field work (92.1% salary, 7.9% benefits); 50% FTE summer each year for 2 years	\$ 8,990
<b>Equipment/Tools/Supplies:</b>	
One water quality meter (YSI) purchased in year 1 for monitoring on experimental ponds (Year 1) and water chemistry measures in wetlands sampled (Year 2)	\$ 1,500
Experimental Ponds: Set-up supplies in Year 1, including necessary replacement/updates to existing system: covers, aeration tubing, nets, food (\$1,594); temperature monitors (\$1,313); carbon filters to clean wastewater (\$1000)	\$ 3,907
Experimental Ponds: Water sample supplies for collection from Experimental Ponds in Year 1, including sample vials (\$250/case); filters and syringes to stabilize water samples (\$620/case); standards for water quality (\$30)	\$ 900
Experimental Ponds: Frog specimen collection, preservation, dissection, and histological analysis supplies for Experimental Ponds in Year 1, including collection vials (\$500/case); chemical reagents for euthanasia and preservation (\$609); dissection and histology supplies (scalpels, tissue prep, staining and clearing agents, slides and cover slips, microtome blades) for assessment of internal frog abnormalities (\$1,859)	\$ 3,956
Experimental Ponds: Perfluorooctane sulfonate (PFOS) analysis supplies for Experimental Ponds in Year 1, including reagents and materials to process 100 samples (50 experimental ponds, each with 2 samples, \$1,600 per set of 25 samples)	\$ 6,400
Field Sampled Wetlands: Supplies to collect water samples from wetlands in Year 2, including sample vials (\$250/case); filters and syringes to stabilize water samples (\$620/case); standards for water chemistry (\$15)	\$ 885
Field Sampled Wetlands: Perfluorooctane sulfonate (PFOS) analysis supplies for water samples from vernal pools and prairie wetlands in Year 2, including reagents and materials to process 80 samples (40 wetlands, each with 2 samples, \$1,600 per set of 25 samples)	\$ 6,400
<b>Travel:</b>	
Travel for Experimental Ponds: 2 people, 5-10 day trips to find and collect frog eggs, \$518 mileage (\$0.575/mi * 90 miles RT * 10 trips), \$100 truck charges (\$10/day * 10 days)	\$ 618
Travel for collecting water samples from vernal pools and prairie wetlands: 2 people, 12 days (3 local, 9 greater than 100 miles), \$1,725 mileage (\$0.575/mi * 250 miles avg RT * 12 trips), \$120 truck charges (\$10/day * 12 days), \$1,638 per diem (\$90/night shared room + \$46/day*2 people for meal cost * 9 nights)	\$ 3,483
<b>Additional Budget Items:</b>	
Printing of outreach educational materials (250 2-sided glossy color brochures at \$0.60 each) in Years 1 and 2	\$ 300
100 hours GIS Lab service time to identify field sample locations (\$4.10/hour) in Year 2	\$ 410
<b>TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REQUEST =</b>	<b>\$ 250,954</b>

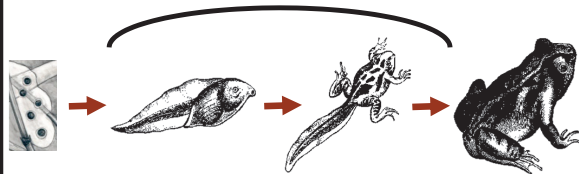
### V. OTHER FUNDS

<b>SOURCE OF FUNDS</b>	<b>AMOUNT</b>	<b>Status</b>
<b>Other Non-State \$ To Be Applied To Project During Project Period:</b>	N/A	
<b>Other State \$ To Be Applied To Project During Project Period:</b>		
If awarded, NRRI will contribute with time/effort as needed for successful completion of project without requesting further funds from LCCMR for the following: Jennifer Olker, Project Manager, additional time to each year of the project. Funded by other NRRI sources, which can be used to Foregone F&A funding of 52% of MTDC (TDC less grad student tuition & fringe: TDC=\$250,951-\$35,692)	\$	Pending
	\$ 111,933	Secured
<b>In-kind Services To Be Applied To Project During Project Period:</b>		
Natural Resources Research Institute: mesocosms @ \$3,100 (equipment owned by NRRI that will be utilized for this research)	\$ 3,100	Secured
<b>Funding History:</b>	N/A	
<b>Remaining \$ From Current ENRTF Appropriation:</b>	N/A	

## Artificial Pond Experiment

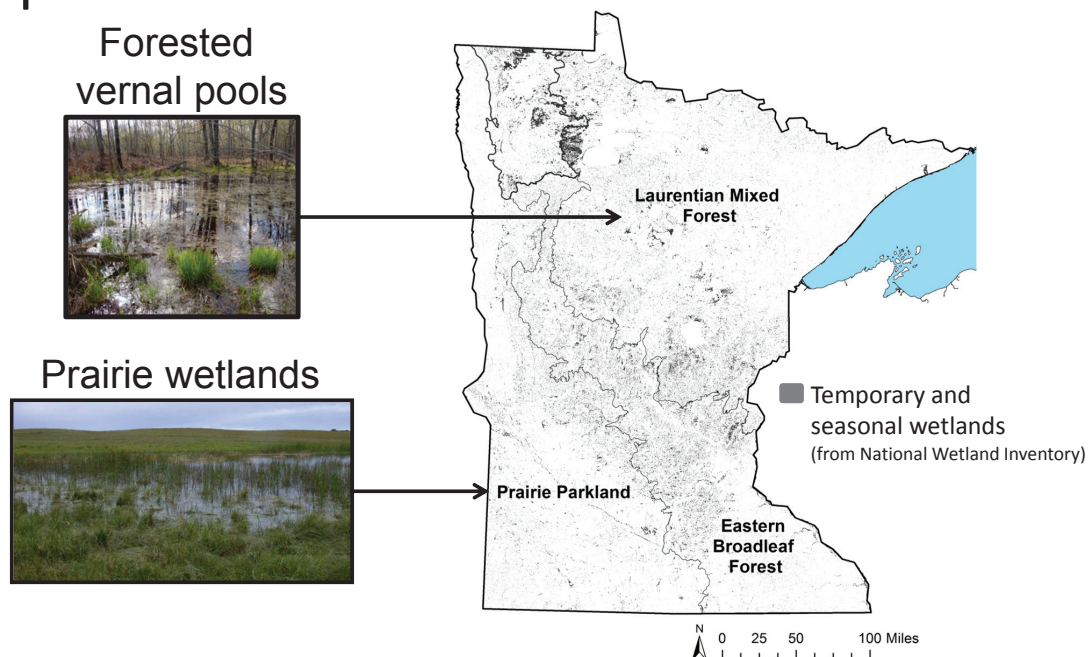


Perfluorochemicals +  
nitrate + accelerated  
drying



- Survival
- Growth and development
  - Skeletal and gonadal abnormalities

## Perfluorochemical contamination in isolated depressional wetlands across Minnesota?



**Project Manager Qualifications & Organization Description**

Dr. Jennifer Olker is a Research Associate at NRRI, University of Minnesota Duluth (UMD). Dr. Olker has over 10 years of experience in amphibian and wetland ecology and specializes in amphibian ecotoxicology, including mesocosm exposure experiments, developmental abnormalities, and gonadal histology. Dr. Olker was key personnel in the establishment of the NRRI outdoor experimental pond system, which she used for multiple exposure experiments with native frog species (as a component of her dissertation research). Dr. Olker is the Principal Investigator/co-investigator for aquatic ecology and ecotoxicology projects: Coastal wetland Vulnerability and Impact Assessment: Climate Change Impacts of Coastal Planning (funder: USGS); Evaluating Vital, Small Forested Wetlands (funder: Minnesota Lake Superior Coastal Program); Gonadal Deformities in Smallmouth bass as Indicators of Endocrine Disruption in the St. Louis River Estuary (funder: Minnesota Sea Grant). Through these and other projects, Dr. Olker has 5+ years managing project budgets and deliverables.

Co-investigators provide specialization in food web effects, functional changes in response to anthropogenic stress, and ecological statistics (Dr. Katya Kovalenko) and organic chemistry (Dr. Igor Kolomitsyn).

Dr. Kovalenko has experience with toxicology assessments of heavy metal compounds and led an overview of naphthenic acid toxicity as a part of a comprehensive assessment of food web structure in reclamation of oil sands process materials (*Ecological Applications*, 2013). She has 17 publications in peer-reviewed journals and serves as an editor for two aquatic ecology journals (*Hydrobiologia*, *Neotropical Ichthyology*).

Dr. Kolomitsyn has over 15 years of experience in organic chemistry and technology, which includes the following directions of organic chemistry: organic chemistry of cage structures and terpenes, fatty acids, synthesis of biologically active compounds, chemical modification of natural products, and chemistry of chemical extractives, chemistry of peracids and peresters and mechanisms of organic reactions. Dr. Kolomitsyn has authored 13 papers in national and international organic chemistry journals and has seven United States patents.

The **Natural Resources Research Institute (NRRI)** is a part of the University of Minnesota Duluth. NRRI's mission is to promote private sector employment based on natural resources in an environmentally sensitive manner. NRRI scientists have extensive experience in applied ecological research on terrestrial and aquatic systems.

- The NRRI outdoor experimental pond system has been used for multiple exposure experiments and is available for the proposed project at no charge.
- The primary goal of Dr. Kolomitsyn's laboratory is to deliver R&D support to private sector through technology development and technology transfer. The primary focus is a development of new waste water treatment technologies as well as a development of new materials. The laboratory is fully equipped to execute experimental protocols related to chemistry of water as well as organic chemistry. Recently, Dr. Kolomitsyn's laboratory developed, patented, and licensed new peat granular sorption media, which was designed to adsorb poisonous heavy metals from mine water.
- The Natural Resources Geographic Informational Systems (NRGIS) laboratory at NRRI was established in 1988 through a National Science Foundation grant and matching funds from the University of Minnesota. The NRGIS lab provides NRRI and other university researchers with access to advanced GIS methods for applied and basic natural resources research, derives new research methods in spatial analyses, and serves data to public and private agencies to fulfill public research and education needs. The lab provides the equipment, software, and computing power needed to analyze large spatial datasets including the National Wetlands Inventory, LiDAR data, land cover/land use data, and aerial photography.