**Project Title: Next generation solutions to ensure healthy water resources for future generations**

**John P. Giesy** (PI), Canada Research Chair in Environmental Toxicology, Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, john.giesy@usask.ca (molecular toxicology, eco-genomics, monitoring, ultra-high-resolution mass spec).
**Mark R. Servos** (Co-I), Canada Research Chair in Water Quality Protection, Department of Biology, University of Waterloo, mservos@uwaterloo.ca (ecotoxicology, biomonitoring).
**Paul D. Jones** (Co-I), Northern Ecosystem Toxicology Initiative Chair, School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan, paul.jones@usask.ca (analytical chemistry, fish ecology, eco-genomics).
**Markus Hecker** (Co-I), Canada Research Chair in Predictive Aquatic Toxicology, School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan, markus.hecker@usask.ca (environmental omics, eDNA, sequencing, fish biology).
**Timothy Jardine** (Co-I), Associate Professor, School of Environment and Sustainability and Toxicology Centre, University of Saskatchewan, tim.jardine@usask.ca (ecology, ecosystem structure and function).
**Bram Noble** (Co-I), Professor, School of Environment and Sustainability, University of Saskatchewan, b.noble@usask.ca (environmental planning, assessment and policy).
**Paul Craig** (Co-I), Assistant Professor, Department of Biology, University of Waterloo, pcraig@uwaterloo.ca (epigenetics, fish physiology, toxico-genomics).
**Barb Katzenback** (Co-I), Assistant Professor, Department of Biology, University of Waterloo, bkatzenback@uwaterloo.ca (environmental immunology, environmental stressor interactions).
**Andrew Doxey** (Co-I), Assistant Professor, Department of Biology, University of Waterloo, acdoxey@uwaterloo.ca (bioinformatics, genomics).
**Collaborators: Vince Palace**, IISD-Experimental Lakes Area (ELA), vpalace@iisd-ela.org (mesocosms, studies at ELA); **Xiaowei Zhang**, State Key Laboratory of Pollution Control and Resources Reuse, School of the Environment, Nanjing University, Nanjing, China (world expert in DNA-barcoding for environmental assessment and is directing the Jiangsu Provincial program of monitoring aquatic systems in China).

**Table 1. List of Partners and Collaborators and their Roles/Responsibilities**.

| Category | Partners (Key contact) | Role |
|---|---|---|
| Federal Agencies | 1. Agriculture and Agri-Food Canada, Catherine Champaigne, catherine.champagne@canada.ca | Participate as advisors, providing access to data and samples (in-kind estimated as $15K) |
| First Nations and Metis | 2. Weylin Bomberry, Six Nations of the Grand River, (519) 445-0330, weylinbomberry@sixnations.ca | Support for sample design and collection, integration of Traditional Environmental Knowledge. |
| Provincial Ministries and Agencies | 3. Alberta Agriculture and Forestry, Jannelle Villeneuve, janelle.villeneuve@gov.ab.ca | Provision of samples from biomonitoring program including remote sites plus technical input and sharing of data, specifically for irrigation water sources (in-kind estimated as $75K) |
| | 4. Alberta Environment and Parks, Chief Scientist, Fred Wrona, Fred.Wrona@gov.ab.ca | Provision of samples and data from sites monitored and investigated during ongoing monitoring programs. Provide advice on biomonitoring and access to historical monitoring data. (in-kind contribution estimated as $25-50K) |
| | 5. Saskatchewan Ministry of the Environment: Fish and Wildlife Branch (Matt Tyree: matt.tyree@gov.sk.ca; phone: (306) 953-2675) | Collection of samples in remote locations; assistance in project design and student advising |
| | 6. Ontario Ministry of the Environment Conservation and Parks (OMECP), Sonya Kleywegt Sonya.Kleywegt@ontario.ca | Access to monitoring data, participation in meetings, site visits and advice on applications. |

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| | 7. Ontario Ministry of Natural Resources and Forestry (OMNRF), Ken Cornelisse, ken.cornelisse@ontario.ca (519) 830-0822 | Support the extension of the research into additional sites, such as Mill Creek and McKenzie Creek, provide historical data (in-kind contribution estimated as $4.5K) |
| Watershed authority and councils | 8. Grand River Conservation Authority, GRCA Crystal Allan, Supervisor, Natural Heritage, callan@grandriver.ca | Provide staff time for coordination of meetings and access to monitoring data. GRCA represents and coordinates numerous watershed stakeholders (in-kind contribution estimated as $30K) |
| IISD-ELA | 9. Experimental Lakes Area, Chief Research Scientist, Vince Palace vpalace@iisd-ela.org | Access to historical data sets for the ELA sites, participation of scientists in studies, access to ELA lakes and some costs for staying at ELA (in-kind contribution estimated as $99.6K) |
| Industry | 10. Orano Resources Canada Inc., Arden Rosaasen, Manager, Environmental Science, arden.rosaasen@areva.ca; phone: (306) 343-4659 | Logistics support, access to historical databases for biomonitoring/EEM, participation in meetings and advice (in-kind contribution estimated as $100K) |
| NGOs/ community groups | 11. Trout Unlimited Canada, Alex Meeker, Ontario Provincial Biologist | Attending meetings, provide fish data, advice based on local experience and support for sampling. (in-kind contribution estimated as $6K to 10.5K) |
| NGOs/ community groups Institutional | 12. Middle Grand Chapter of Trout Unlimited Larry Halyk, lhalyk1837@gmail.com; 226-821-1245 | Provide logistical support, experience/advice, data on local streams and opportunities for outreach |
| | 13. Friends of the Grand River, Rob Voisin, Board Chair, robvoisin@friendsofthegrandriver.com | Provision of advice and review, participating in collecting samples, and supporting education/outreach and supporting knowledge exchange among members. |
| | 14. Ontario Federation of Anglers and Hunters, Tom Brooke, tom_brooke@ofah.org | Access to membership and support for dissemination of outcomes. |
| | 15. rare Charitable Research Reserve, Jenna Quinn, Jenna.Quinn@raresites.org | Provision of advice, background information and historical data, participate in meetings, coordinate volunteers and manage site access (in-kind contribution estimated as $ 4.5K) |
| | 16. Southern Ontario Water Consortium (analytical and ecotox nodes), Brenda Lucas, brenda@sowc.ca | Access to advanced instrumentation and data platforms |
| International | 17. Tai Lake riverine pollution prevention and management office, Chinese Ministry of Science and Technology China Prof. Hongxia Yu, yuhx@nju.edu.cn | Applying eDNA in env monitoring and management in China; exchange students and PDFs between China and Canada. Estimated in-kind support for travel and collection of data and support of students in China developing joint methods. $200K |
| International | 18. Nanjing University, Prof. Xiaowei Zhang, howard50003250@yahoo.com | Developing and applying statistical analyses for use of eDNA data. Estimated in-kind support for exchange of students, methods and joint publications $100K |
| | 19. RECETOX, Masaryk University, Klara Hilscherova, hilscherova@recetox.inuni.cz | Developing and applying statistical analyses for use of eDNA data. Estimated in-kind support for exchange of students, methods and joint publications (in-kind contribution estimated as $200K; leveraged $1,600K) |
| | 20. Melimoyu Ecosystem Research Institute (MERI), Gustavo Chiang, PhD, Scientific Director, Av. Kennedy lateral 5682, Vitacura-Santiago, Chile +56 2 29535192 | Assist with field sampling, access to lab facilities for processing and preservation of samples. |
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**Summary:** During Phase 1 (Years 1 and 2) of the proposed 7-year program we have made significant progress in developing targeted (qPCR) and non-target (next generation sequencing [NGS] based barcoding and metabarcoding) eDNA approaches to monitor current status and predict future trends of structures and functions of environments exposed to stressors in Canada. We have demonstrated the utility of these transformative technologies, especially for rapid monitoring to determine absence or presence of species and relative changes in abundances. We have compared results of environmental DNA (eDNA) with those based on conventional taxonomy in a variety of field and semi-controlled biomonitoring programs and controlled exposures to known intensities of chemical stressors. We have partnered with industries and provincial agencies to demonstrate where eDNA technologies can be used in monitoring and surveillance programs to make them more rapid and increase performance over broader areas for less cost. During Phase 2, by continuing to work with previous and additional collaborators, we will be able to better define parameters, such as water quality, hydrology, etc., that influence interpretation of results. A systematic review of existing biomonitoring and bio-assessment programs carried out by agencies, industries and community groups, and underlying Acts and Regulations that support them, has allowed us to better define how new technologies can be incorporated and support end-users. The research team is a strategic assemblage of nationally and internationally recognized experts and emerging researchers that have forged strong working relationships. We have recruited a very strong cadre of postdoctoral fellows and Ph.D.- and M.Sc.-level graduate students who will continue in Phase 2, that includes representation of gender and diversity. We are proposing to continue in Phase 2 as an established team to apply the methods developed and validated during Phase 1 to work with partners to continue comprehensive demonstration projects that include various types of ecosystems, representative of Canadian water resources; large northern rivers/deltas and boreal lakes, as well as more southerly areas with urbanizing watersheds. The research will center on well-characterized environments at the Experimental Lakes Area (ELA), instrumented watersheds (e.g. Grand River), lakes, rivers and ponds in Saskatchewan and irrigation districts of Alberta, that are undergoing rapid changes due to changes in climate and inputs of nutrients and crop protection chemicals. The project will validate techniques under controlled conditions (e.g. laboratory experiments and mesocosms), build eco-genomic databases for key organisms and environments, and support applications of technologies to address needs of end-users for improved and more effective bio-assessment/monitoring including Canada's Environmental Effects Monitoring Program (EEM) under the Fisheries Act. eDNA will be used to detect and predict changes in ecosystems across trophic levels. These techniques will be transferred to cooperators to support long-term goals to assess aquatic resources in support of end-user needs and priorities at the local, provincial, and national levels. The program will be conducted in close cooperation with local, provincial, federal, industry and non-government organizations (NGOs), with whom we have developed strong working relationships. While the Phase 2 program will build upon existing relationships, several new collaborators have been added to help develop capacity to assess biological structure and function as well as status and trends at the ecosystem and watershed levels. eDNA approaches have the potential to be applied across the GWF platform and support studies in northern watersheds (e.g. NWT), Laurentian Great Lakes and tributaries, urban watersheds, and alpine lakes and rivers. As part of our activities we are collaborating with researchers in Jiangsu Provence, China where eDNA is being applied as part of routine environmental monitoring and is accepted from regulatory purposes. We will also be collaborating with researchers at Masaryk University in the Czech Republic where they are initiating a program to apply eDNA for monitoring of rivers. Prof Giesy has been appointed as the chair of the advisory panel for the TEAM project and will be making annual trips to Brno to coordinate these activities. We will also apply methods to assess biodiversity in remote, pristine rivers in Patagonia in collaboration with the MERI Foundation.

**Rationale Showing Alignment to User/Stakeholder Needs and GWF Vision**: Phase 2 of the program will continue to provide end-users with new and better tools for assessing effects of stressors, both natural and those caused by humans, such as changes in land use, climate, agricultural runoff and wastewater effluents, on health and resilience of ecosystems. For example, agencies such as the Saskatchewan Ministry

of the Environment (MoE), Fish and Wildlife Branch, have expressed strong interest in emerging technologies, such as eDNA, for detection of endangered or invasive species and they seek to create a regional strategy, involving SK, MB, AB, BC, YK, to advance application of eDNA in surveillance since it can detect such species much earlier than traditional field ecological approaches. In Phase 1 we developed and applied a strategy to monitor for zebra mussels, an invasive species in Manitoba and Saskatchewan. We have successfully transferred the technology to the Saskatchewan MoE so they or their contractors can continue to apply the technology for monitoring and early detection. Other agencies, such as Ontario Ministry of the Environment Conservation and Parks and the Grand River Conservation Authority have also expressed interest to apply the technology in their monitoring programs. Because of the remoteness of sites and the need to monitor, the Resource Management and Compliance Division of MoE, has also identified a need for improved monitoring in its Boreal Watersheds Monitoring Program. We successfully applied eDNA technologies for monitoring for presence of wood frogs in remote areas of Saskatchewan and at the rare Nature Reserve in Ontario. In urbanizing environments, such as the Grand River in southern Ontario and Wascana Creek in Saskatchewan, multiple stressors have changed habitats and water quality leading to changes in structures and functions of these ecosystems. Further end-user groups with whom we have partnered use the Grand River and contributing tributaries for both recreation and research that align well with implementation of eDNA as an alternative for species documentation and diversity assessment, characterization of their spatial and temporal movement, and invasive species identification. Organizations charged with managing these watersheds, such as the Grand River Conservation Authority (GRCA) and Ontario Ministry of Natural Resources and Forestry (MNRF), are struggling to assess ecosystem integrity and resiliency in the context of natural variability and cumulative effects. In addition, habitats of cold-water species, such as brook trout, are particularly threatened by climate change and urbanization in southern Ontario. The Six Nations of the Grand River have an interest in applying eDNA as an additional tool in assessing biodiversity on their territorial and traditional lands. The rare Charitable Research Foundation and the Melimoyu Ecosystem Research Institute (MERI) are both interested in assessing biodiversity in their established nature reserves by use of eDNA. We will continue to partner with these groups and expand the repertoire of species that these technologies can help them detect and monitor. Many threatened, endangered, and invasive, aquatic species occur in watersheds and their low density makes them particularly difficult to assess, especially in remote areas. Assessing changes in aquatic communities at watershed scales (e.g. ecological gradients) is difficult and new tools with more comprehensive approaches such as the here-developed eDNA technologies are needed. Development of temporal and spatial measures of eDNA will support biomonitoring efforts related to a variety of programs such as Canada's EEM that underpin regulatory development for resource extraction, other industries and municipal wastewaters. We are also partnering with the research team at Nanjing University that is coordinating application of eDNA technologies in on-going monitoring programs of Jiangsu Province, China. We are also partnered with the Research Center for Chemistry and Toxicology (RECETOX) of Masaryk University to develop eDNA technologies for monitoring of rivers in Europe. They have sent faculty and students to U of S to learn how to collect and process samples. This year we will send faculty and students to the Czech Republic to implement eDNA.

This proposal directly supports the GWF's overarching goal to deliver solutions for management of risks due to local activities of humans and changes in climate, informed by cutting-edge water science, to address water futures in cold regions. The research proposed during Phase 2 will continue to address a number of key pan-Canadian and international needs. An enhanced ability to monitor ecosystems is paramount to assessing changes sufficiently early so that effective remedial actions or adaptations (e.g. best management practices, treatment) can be developed and implemented to protect aquatic ecosystems, and therefore, the services they provide to society. Early warning of change will allow communities to "strategically adapt, manage, and balance their water needs", and will be critical to addressing current and future "social, economic and environmental challenges". These approaches will allow risk managers to document

recoveries of affected ecosystems in an efficient and objective manner. While being applicable globally, the methods proposed are particularly appropriate for Canada's environments that can be remote and, thus, more difficult to sample and assess. As a new tool in the "toolkit", use of eDNA can inform development and application of adaptation options that build resilience in systems to prepare for future hydro-climatic scenarios, ensuring protection of ecosystems and their sustainability. In particular, the proposed work will integrate cumulative interactions of both biotic and abiotic stressors and determine how cumulative effects can be monitored, managed, and mitigated through application of emerging technologies. The program is transdisciplinary, pan-Canadian, and delivers transformational science and spans representative Canadian watersheds in the north, west (prairies) and more urbanized southeast. The "big data" generated through next-generation sequencing and measurement of eDNA creates unprecedented opportunities to holistically assess ecosystems in near real-time, but it requires complex bioinformatics and computational capacities. However, methods for collection of samples are potentially more straight forward and robust than traditional taxonomic approaches, making these techniques applicable to routine monitoring and possible involvement of local communities in sampling programs. Our research will make these emerging tools more accessible to a broad audience, including various GWF researchers. While our research is focused initially on Canadian ecosystems, the approach is of global applicability, which will improve the abilities of diverse jurisdictions to rapidly and cost-effectively monitor status and trends in ecosystem health in rapidly changing aquatic environments. The research team consists of a diverse, interdisciplinary group of leading experts that are at the forefront of applying such advanced technologies in environmental bio-assessments nationally, as well as internationally, and will therefore place GWF research on a global stage. We are also working collaboratively with a groups in China, Chile and Europe.

**Objectives and Methodology (Phase 2):** We have refined the subprojects to advance the objectives in Phase 2 to improve reliability of the methods, including fates of eDNA, validation of eDNA against traditional sampling techniques and application at demonstration sites in support of collaborators and end users. Traditional monitoring by agencies to assess status and trends of change in aquatic ecosystems can be limited by use of traditional taxonomic approaches, based on morphology, that are labor-intensive, costly and often have a slow turn around time, and therefore usually only allows for assessments with limited spatial or temporal resolution. Advances in next-generation 'omics technologies, computational capacities, bio-informatics and access to online resources/ databases provide a promising path forward to combine these emerging techniques with more traditional approaches to improve their resolution, throughput and accuracy. The primary objective of Phase 2 of the proposed 7-year research program is to apply targeted (qPCR) and untargeted (NGS) eDNA methods developed during Phase 1 to: 1) assess absolute and relative changes in populations and communities in aquatic environments of northern and urbanized Canadian watersheds, and 2) characterize these communities in surface waters and sediments as indicators of ecosystem health/ change under varying degrees of natural and anthropogenic stressors.

Now that we have developed a genetic library of endemic and invasive species, eDNA can deliver a high level of taxonomic specificity without need for labor-intensive collections and identifications of individual organisms. Furthermore, these technologies are capable of detecting as little as a single molecule of DNA; or a single organism in a stretch of river, pond or lake. However, until recently, application of eDNA analyses as a molecular fingerprinting technique has been limited because of the time-, labor-, and cost-intensive nature of traditional, low-throughput sequencing techniques. Development of NGS methodologies, such as the Illumina sequence-by-synthesis technology, recently procured by the Global Water Futures (GWF) program, in combination with advanced bioinformatics tools have laid the foundation for novel, highly efficient, high-throughput approaches for eDNA analyses of complex biological communities [1, 2]. While such environmental metagenomics approaches have been used previously to demonstrate effects of environmental stressors, such as metals and changes in land-use, on prokaryotic communities in freshwater [3, 4], assessment of larger ecological effects of multiple anthropogenic stressors on eukaryotic communities using eDNA is a relatively recent and novel approach. Aquatic communities are

complex, with unicellular to multicellular organisms, including algae, insects, crustaceans, and vertebrates. Among these assemblages, periphyton, such as diatoms, algae, cyanobacteria, and benthic macroinvertebrates are primary constituents, and have been used as indicators of effects of stressors, including chemicals, on ecosystems [5]. Due to diversities of aquatic communities, parallel biomonitoring of multiple assemblages is limited by the need to collect, identify, classify, and enumerate taxa using traditional, visual methods of taxonomy based on morphometric characteristics. For this reason, individual scientists have historically focused on particular taxonomic groups, in which they had expertise for identification. Despite resolution of taxonomic approaches based on morphology, recently developed eDNA metabarcoding methods provide more powerful tools for fine-scale monitoring of biodiversity in ecosystems [6-8]. Some ecological components, including benthic microbial communities, have been neglected in conventional assessments of aquatic ecosystems [9-11]. Studies on linkages and shifts in structure and function of aquatic communities using traditional censuses of zooplankton and vertebrates, including fishes, have been widely conducted but few have compared results to eDNA analyses. Environmental DNA has potential to provide more complete descriptions of taxa present and do it more quickly and affordably than more traditional methods. During Phase 1, eDNA libraries were developed for both microbial and macro-biological communities that will allow comparisons of eDNA studies to results of studies based on more traditional methods for identifying "species". Operational taxonomic units (OTU) can also be assigned Latin names to facilitate these comparisons. For instance, one of the Co-Is (JPG) and collaborator (Zhang) have been applying eDNA (metabarcoding) in Tai Lake and the Yangtze River in China, and DNA-barcoding has been included in monitoring programs by authorities of Jiangsu Province, China (see letters of collaborators). Comprehensive studies comparing results of high-throughput NGS-based metagenomics to more traditional measurement endpoints to assess structure and function of whole food webs in northern ecosystems of Canada are rare [12]. Establishing, validating, and applying an end-user driven toolbox will be accomplished through four main sub-objectives in collaboration with continuing and new partners.

**Specific Objectives for Phase 2:** During Phase 1 of the project much of the effort was to develop and document methods for detection of environmental DNA in freshwater ecosystems and codifying Standard Operating Procedures (SOPs). A total of 44 SOPs established and made available to collaborators and end users. Phase 2 will continue ongoing studies initiated with partners during Phase 1 and focus on validation and application of these approaches in support of advancing current bioassessment and biomonitoring activities. It was established, through a survey of end-users, that the proposed research in Phase 2 is critical for adoption of eDNA by project partners and other end-users. Objectives presented below are consistent with the original proposal, but also build on the progress to date, address emerging questions and optimize opportunities for knowledge mobilization.

**Objective 1.** ***Improve reliability, predictability and interpretation of detection of eDNA using lab studies and field manipulations.*** Environmental DNA is obtained through collection of samples from the environment, which contain DNA fragments originating from whole organisms, or shed and decayed cells of all of the inhabitants in a particular aquatic system. While this method appears straight forward, presence/abundance and rates of release by aquatic organisms can vary depending on factors such as type of taxa and size [13], life stage [14], and organismal health [15] as well as environmental conditions. Abiotic and biotic factors, such as temperature, flow, pH, and UV light intensity can affect persistence of eDNA in water [16-19]. Persistence of eDNA can range anywhere from 0.9 to 54 days [13, 14], indicating that an appropriate timeframe, as well as environmental and biologic conditions must be considered to ensure that appropriate samples are taken so that they can be reliably interpreted. Studies will be conducted under controlled conditions or field based manipulations employed in several studies (described in the sections

below) to assess the ability and reliability of the eDNA technologies to detect and predict species compositions under a variety of environmental conditions.

The form and structure of eDNA captured on filters during sampling events is currently not well characterized, and it is therefore difficult to predict its fate. A series of experiments will be conducted where specific sequences of "naked" DNA (or modified on other carriers to change the properties, e.g. nanoparticles) are added to water samples under controlled lab conditions (e.g. water quality, light, temperature). In parallel field experiments, specific synthetic DNA sequences will be added to streams to determine its degradation, fate and distribution while simultaneously examining the naturally occurring eDNA in the water of the same samples (**UW PhD 1**). Note these studies can be conducted at the same time as other collections, therefore adding considerable understanding of the processes controlling persistence and distribution of eDNA in natural environments. DNA has been used as a hydrologic tracer in streams and groundwater [20-22]. This novel approach, potentially using essentially an unlimited number of unique tracers simultaneously, has many potential applications in other GWF projects. An initial proof of concept is being conducted in Alder Creek in the fall of 2019. Linked to other field studies, controlled additions/manipulations will be conducted to contrast the fate in natural systems compared to those in controlled or manipulated systems (**UW PhD 1, 2**). Specifically during Phase 2 we will: 1. Study forms and structures of DNA collected using various sampling and collection procedures developed during Phase 1, including filtration and sedimentation that are described in detail in our SOPs; 2. Determine rates of degradation and transport of eDNA in natural ecosystems and controlled field and laboratory mesocosm studies using spiking with specific amounts and types of DNA; and 3. Explore with other Pillar 3 subprojects the option of using eDNA as possible hydrological tracers. Specifically, methods currently applied to isolate eDNA for targeted analysis (e.g. brook trout, *Dreissena spp*, Prusian carp) lack a good internal standard to allow assessment of the quality and integrity of the recovered eDNA (as is common in trace analytical chemistry). Although several approaches are typically applied, one approach to ensure that isolation of eDNA is properly conducted is spiking water samples with a known amount of DNA (or modified particles labeled with DNA) that is not present in the surveyed environment (e.g., synthetic DNA sequence) prior to collecting and extracting eDNA. Synthetic DNA can potentially be used as a proxy to 1) determine extraction efficiency; 2) set equal template amounts in the qPCR assays; and 3) inform on relative abundancies of target organisms in environments. We will examine if a set of synthetic standards can be developed to help interpret and test for integrity of eDNA samples.

**Objective 2.** ***Validate eDNA methodologies by comparing results to conventional taxonomic and ecological methods in aquatic environments.*** Bioassessments can be complex, time consuming and expensive. If eDNA technology is going to be employed by agencies and institutions, especially for regulatory purposes, it must be validated and a link between the "new" technology established with traditional approaches currently applied in biomonitoring. We therefore are applying the eDNA approach in a number of key experiments that are/ will be integrated with ongoing biomonitoring programs and research efforts by our project partners. This will enable us to leverage resources to a maximum, while also supporting our partners' goals. Also, by demonstrations designed around programs of interest and importance to the partners we will gain greater trust and credibility for the approach. We will contrast taxonomic data obtained with conventional approaches to results obtained using eDNA (targeted qPCR-based and non-target DNAseq-based studies). We have selected a number of sites where there are considerable leveraged data sets or ongoing research programs ranging from natural lakes and streams, impacted sites and manipulated ecosystems.

***2A. Natural variation in lakes and streams of the Experimental Lakes Area (ELA).*** In the ELA, a wide range of lentic environments have been monitored for populations and communities of phytoplankton, zooplankton and fish for more than 50 years. Some of these lakes were experimentally manipulated in the past (e.g. nutrients, pharmaceuticals and mercury). These monitoring activities have been or are occurring

on a continuous basis, some monthly, some quarterly and some annually. We have guaranteed access to all of the archived monitoring data and that being collected currently (see letters of support) and will compare results of eDNA measurements with the data collected currently and historically by ELA staff using traditional taxonomic methods. This is an extensive data set and an internationally recognized research facility that provides a unique opportunity to use historical data to develop a library for use in eDNA barcoding and using current and archived data to calibrate and validate both the targeted and untargeted eDNA methods developed during Phase 1 of this project **(US PDF 1**, **US PhD 1)**

***2B. Environmental gradients in the Grand River sub-watersheds (Bauman Creek, Mill Creek, Alder Creek).*** Biodiversity of fishes, across an environmental gradient will be assessed in sub-watersheds of the Grand River, including Bauman Creek (on the rare Charitable Research Foundation Reserve), and Alder/Washington Creek (Ontario Water Consortium instrumented watershed), as well as McKenzie Creek (see Six Nations project below), using eDNA metabarcoding and contrasted to traditional methods. Each stream will be characterized using multi-pass electrofishing at multiple sites from the headwaters to the confluence. Each of the streams selected have distinct gradients of environmental conditions, resulting from different groundwater and morphological characteristics (glacial features) **(UW PDF, UW PhD 1, 2)**.

***2C. Biodiversity in Great Plains rivers and boreal lakes.*** Alberta and Saskatchewan have ongoing programs to monitor aquatic environments, especially streams and rivers as well as boreal lakes, including along gradients of potential impacts from industries and urban areas. We will coordinate sampling efforts and share data with agencies in both provinces. We will collect data in each season where monitoring of phytoplankton, benthic invertebrates and/or fishes are being conducted by our project partners, including sites upstream/ downstream of major potential stressors (oil sands activities, municipal wastewater discharges), to determine if shifts in community composition are detectable using eDNA approaches, and whether any potential trends observed are consistent with those determined through traditional taxonomic assessments. Furthermore, through our ongoing partnership with Orano we can generate information to strengthen confidence in applying the newer technology in a regulatory arena of routine Environmental Effects Monitoring (EEM). These EEM surveys involve fish and invertebrate community assessments over several decades, and this considerable knowledge will be applied to calibrate eDNA approaches in support of environmental monitoring programs mandated by the Government of Canada **(US PhD 2). Results of s**tudies being conducted with Orano will be used as test of the utility of eDNA and its' relationship with traditional biomonitoring.

We are partnered with both Alberta Agriculture and Forestry (AAF) and Alberta Environment and Parks (AEP), both of which have ongoing structured programs to monitor status and trends in water quality in various rivers in Alberta. The work with AAF will focus on surface waters used for irrigation and specifically monitor for development of phytoplankton communities. The eDNA data will be used to test the hypothesis that development of hazardous blooms can be monitored and predicted by eDNA. We have an opportunity to compare the use of eDNA for making absolute and relative quantification of various species of phytoplankton, including those that cause hazardous algal blooms.

The partnership with AEP will focus on comparing eDNA to the monitoring of benthic invertebrate communities at indicator sites in a network of rivers spanning a range of ecosystems across the province. We plan to collect eDNA samples synoptically during field sampling of benthic invertebrates.

***2D. Detecting ecosystem change during replicated exposures in mesocosms.*** Studies of controlled exposures to various stressors on phytoplankton, zooplankton and fishes are being conducted in limnocorral mesocosms placed into lakes at the ELA. To compare results of eDNA with more traditional measure of community structure and function, our team has been and will continue to collect samples for eDNA from two studies being conducted in these mesocosms. One of these studies focused on assessing the impacts of a range of concentrations of bitumen and the other study aimed to assess the effects of selenium (Se) on lake communities. Support for taxonomic identification of phytoplankton, zooplankton and fishes present in these environments is being provided by faculty, staff and students at the ELA. This creates an opportunity

to assess how eDNA can be applied to assess changes in aquatic communities exposed to different stressors under semi-controlled conditions where accurate determinations of numbers of a wide range of aquatic organisms can be determined over time. This will not only provide a direct comparison between use of eDNA and traditional taxonomic methods, but also allow control of other factors, such as dilution and exogenous sources of eDNA that can influence interpretation of eDNA. **(US PDF 1** and **US PhD 3)**

**Objective 3.** ***Mobilize knowledge to include molecular approaches (eDNA) in existing biomonitoring and bioassessment programs.*** Assessors and decision-makers require tools that enable them to include information in a usable form that can be tailored to meet their program and decision-making needs [23]. To ensure that the methods and tools developed add value relative to needs of end-users, we have been examining end-user monitoring and decision support needs, as well as identifying pathways for incorporating eDNA (targeted and NGS) approaches in existing monitoring and assessment programs. In Phase 1, we have completed 40 interviews with government and academic scientists, regulators, lawyers, consultants and others involved in aquatic bioassessment that will document end-user perspectives on opportunities and challenges in incorporating eDNA as a biomonitoring tool. As we validate the approaches and assess the likelihood of their uptake by government agencies and industries, we can begin applying them to address end-user needs. We will address how eDNA can add additional interpretative value or be used as an alternative to traditional bioassessments.

***3A. Detecting rare, threatened and invasive species.*** Detecting the presence of invasive and threatened species is critical for management interventions. Minimal spatial distribution data are available for species of special interest because of difficulty of sampling for animals with lesser population densities or patchy distributions [24]. Carrying on from our successful partnership initiated during Phase 1 of this project with the Province of Saskatchewan, we will expand our current work by developing methods and apply them for screening for invasive species including zebra and quagga mussels, Prussian carp, rusty crayfish and spiny water flea in areas of high risk (reservoirs with recreational fishing pressure) such as Lake Diefenbaker and Buffalo Pound Lake in SK. Data generated will be co-generated with our end-user partners, who will incorporate this information into their larger surveillance programs. We will use the data in combination with traditional monitoring techniques to further validate use of eDNA for monitoring status and trends of populations of fishes and other organisms of interest. These sorts of comparisons and applications for real-world programs with provincial agencies are critical to moving the technology from research to application. Personnel with whom we have worked are impressed by the results but want further verification.

Alberta and Saskatchewan's list of species at risk includes the lake sturgeon, bigmouth buffalo, bull trout, west slope cutthroat trout, chestnut lamprey and northern leopard frog. We will develop and apply targeted sequencing of eDNA to detect the presence of at-risk species, and to establish extensive habitat-species maps to identify optimum habitat conditions to aid in their conservation. Working with the Saskatchewan MoE, we will: 1) obtain (develop where needed) and test primers for four aquatic at-risk species found in diverse habitats in the southern prairies, such as amphibians and special fishes identified by collaborators; 2) screen >100 sites per year for two years for the species of interest; 3) collect habitat information on water quality, physical features at these sites; 4) assess associations between habitat variables and species presence using mathematical modeling, including boosted regression trees to identify characteristics of positive association; and 5) create distribution maps and habitat associations and provide them to our partners to enhance management of these species. (**US PhD 3)**.

***3B. Enhancing biomonitoring by use of NGS eDNA techniques.*** Advanced molecular techniques (eDNA-NGS) have the potential to greatly enhance interpretation of existing and mandated environmental monitoring programs by industries. These studies will be focused on annual surveys conducted by Alberta Environment and Parks (AEP) (focused on macroinvertebrate communities in streams in Alberta), Alberta Agriculture and Forestry (AAF) (focused on phytoplankton, including cyanobacteria in irrigation channels

of Alberta) as well as those being conducted by Orano, which cover the complete range, including phytoplankton, benthic invertebrates, zooplankton, and fishes in lentic and lotic environments influenced by their effluents as well as control areas upstream of their effluents. We are also partnering with the City of Saskatoon to examine how advanced molecular tools can improve interpretation of results of ongoing monitoring of phytoplankton populations. Information on concentrations of chemicals and nutrients collected from the ecosystems of interest that are measured by project partners can be used as co-variates to develop predictive models of the status and trends of community structure and function. These activities will explore how to improve application of eDNA as a tool for end-users to enhance their biomonitoring efforts in multiple watersheds and allow informed planning for remedial actions. It is envisioned that the current non-target DNAseq approaches like metabarcoding that enable an unbiased bottom-up screening for any potential DNA molecule present in the environment of interest will be translated into high-throughput, targeted DNAseq approaches like eDNA barcoding that covers key communities of interest. While non-target DNAseq is a powerful approach to inform unbiased environmental screening of communities, it is limited in that it amplifies any DNA molecule in a sample, and thus, poses a challenge from a bioinformatics evaluation perspective due to limitations of existing data bases. Targeted sequencing approaches have the advantage that they are more feasible to conduct, still cover large assemblies of organisms, and are very high throughput and at low cost. Thus, the goal is to continue to develop Canadian Prairies and boreal lakes fish, invertebrate and microbial DNAseq panels that can be customized to any Canadian environment of interest, and which will enable high-throughput (hundreds to thousands of samples at a time) monitoring for relative changes in community structure. **(US PDF 1 and US PhD 2)**

***3C. Partner with Indigenous Nations.*** Current eDNA barcoding and/ or metabarcoding methodology can be applied to monitor any site and species of interest. Our research group has a unique opportunity to collaborate with the communities representing the Six Nations of the Grand River in southern Ontario who expressed concerns about biodiversity of fishes in the watershed crossing the reserve (McKenzie Creek). In addition, they are concerned about assessing "at risk" turtle populations found in the wetlands and tributaries flowing through the reserve. eDNA (meta)barcoding is a non-invasive tool that can be used to monitor in this environment where cultural considerations are important. In addition to its symbolic and cultural values, turtles are indicator species that can monitor health of ecosystems in which they live. In cooperation with Six Nations participants, we will survey an environmental gradient in McKenzie Creek from headwaters to the confluence with the Grand River as well as associated wetlands. We will use traditional knowledge to guide our site selection and priorities, facilitated through collaboration with the GWF project led by Six Nations researchers. **(UW PhD 1, 2)**

***3D. Apply eDNA in international settings.*** There is potential to further develop a long-term international collaboration with the MERI Foundation (Patagonia) and applying eDNA to monitor invasive brown trout distribution in remote areas in riverine systems on the Melymou Nature Reserve. Brown trout is competing with native Chilean fish species for habitat as feeding and spawning grounds. Application of eDNA in areas that are remote and difficult to access or survey using traditional methods is of great importance and can provide information on future action plans regarding invasive species and preservation of native fish communities. Preliminary studies in 2018 demonstrated that eDNA in water samples from these remote rivers could be collected, transported and analyzed to detect brown trout. Metabarcoding will be used to explore the species composition of fish in these rivers and contrasted to species of invertebrates and (native) fish detected in the gut contents of brown trout. Determining the gut content of brown trout can be time consuming and expensive; however, using eDNA metabarcoding for this purpose may be more efficient and can provide information on cryptic species that cannot be found in the digestive tract using traditional taxonomic approaches. The MERI Foundation will facilitate sample collection and logistics at their remote reserve. **(UW PhD 1)**

We will continue collaborative studies with Prof. Zhang's group in Nanjing, China to demonstrate utility of eDNA approaches for monitoring of environmental quality. Prof. Zhang has just been awarded a

project from the government of China to continue development of eDNA methodologies for use in a national program to monitor status and trends in water quality. We will continue our exchange program where faculty and students from the two laboratories in Nanjing and Saskatoon make reciprocal visits to exchange techniques and collect samples. A number of manuscripts are currently under development based on data collected over the last two years. (**US PDF 1 and PhD 3)**.

**General methods and approach**

***Sampling Sites:*** Final, specific, sampling locations have either already been selected or will be selected in consultation with regional and provincial agencies and organizations, as well as local communities including First Nations and NGOs. These locations will include diverse ecosystems including lakes and rivers in Alberta, Saskatchewan and Ontario, and will be located upstream (reference sites) and downstream (stressed sites) of stressor sources including urban areas, mining/ industrial activities (e.g. oil sands operations) and dams. Specifically, candidate sampling locations include the Grand River, ON, the North and South Saskatchewan rivers and the Saskatchewan River Delta, SK, the Athabasca River, AB, Lake Diefenbaker SK - a large prairie reservoir and Buffalo Pound Lake SK -, a shallow eutrophic prairie lake. In addition, we will apply the developed protocols to well-characterized boreal and prairie lakes and streams. These field studies will be supported by controlled and semi-controlled studies at the ELA and Grand River (tributaries). Studies in very well-characterized systems will be useful for developing quantitative measures and determining rates of decay of eDNA (building better models). For example, the Grand River is part of an urbanized watershed which has been extensively characterized from both a chemical and hydrological perspective, with decades of archived data. Application of eDNA sampling synoptic with chemical and hydrological data collection (e.g. instrumented sub-watershed) will provide an invaluable tool for future monitoring and protection of ecosystem structure and function. Extensive data is available in this watershed from our past research, as well as our numerous partners, that can be utilized and contrasted with emerging approaches using eDNA. Moreover, information on habitat utilization (e.g. occupancy, migration, life-stage) of selected species will be collected through conventional means (ecological assessment, taxonomy) and compared to eDNA data collected in an effort to validate this approach, and support the management objectives of the end-users, with whom we have partnered. Similarly, by conducting studies in the ELA as well as selected northern lakes in Saskatchewan currently subjected to EEM by Orano, we will be able to leverage our results against the large amount of standard taxonomic and water quality measurements being conducted on a seasonal basis. Libraries of eDNA sequences have been developed and annotated, by use of sequence-by-synthesis technology and associated bioinformatics, during Phase 1 of this project. In Phase 2, these technologies will be applied to continue assessing samples from previous and additional locations. Other collections will be focused on particular locations where invasive species and species at risk are considered to be an issue. While testing in controlled laboratory settings and a local urbanized watershed will provide extensive data and validation of the technique, further investigation into the global application of eDNA is essential prior to implementation. Working with our collaborators, these techniques will be applied in mesocosms treated with inorganic Se or diluted bitumen as well as gradients of well characterized areas in the ELA. Finally, we will investigate additional field sites in both the Saskatchewan and Athabasca river systems. This approach will provide end users with more comprehensive and reliable assessments of aquatic resources and ecosystems and form the basis for better assessments and better informed decisions.

Both surface water and sediments will be collected for biological and chemical characterization. In addition, microbial communities (bacteria, periphyton, fungi and protists) and macro-invertebrates and vertebrates and aquatic macrophytes will be sampled using conventional ecological collection methods for taxonomic characterization. Water samples will be collected below the surface by grab sampling (3.5 L). Samples will be filtered (1.5 L) on site using sterile, one-way filters with a pore size of 0.45 μm and 2 L will be preserved by adding 1 mL of chloroform and stored in amber bottles at 4°C for later chemical analyses. Surface (top 5 cm biotic zone) samples of sediments will be collected by use of a Van Veen grab

sampler for characterization of micro- and macro-fauna communities. Samples will be pooled, and homogenized, and an approximately 40 mL aliquot of each will be stored in two sterile 50 mL tubes for archiving for analyses of chemical residues (one for organic and one for inorganic residues). A second 40 mL sub-sample will be snap frozen and maintained at -80°C for molecular analyses. The remainder of the sample (approximately 2 L) will be stored in a plastic bag and frozen at -20°C for measurement of sediment quality parameters including grain size, total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP). pH and redox potential will be determined *in situ* at each site in proximity to the site where grab samples were collected. Chemical and physical characteristics, including inorganic and organic contaminants will be characterized by use of instrumental techniques already available in the Giesy-Jones laboratories. These include use of liquid chromatography and gas chromatography coupled with mass spectrometry invested in by GWF (**US PhD 2).**

***DNA extraction and sequencing:*** Techniques for extraction, sequencing and quantification of DNA have been developed and validated during Phase 1 of this project by our research team for prokaryotes and eukaryotes covered in a total of 44 SOPs developed and posted online (https://gwf.usask.ca/edna/resources/standard-operating-procedures.php). DNA will be extracted from a 0.25 g aliquot of a homogenized sediment or 0.5 L water sample with the MoBio Power Soil DNA kit (MoBio Laboratories, Inc.: Carlsbad, CA, USA) or Qiagen Tissue DNA Isolation kit (Hilden, Germany) respectively and stored at -80 °C [25]. Amplicon libraries will be constructed through PCR amplification of ribosomal and mitochondrial gene products for a limited number of samples, as applicable for the taxonomic group of interest (e.g. 16S for bacteria, 18S for protists, mitochondrial CO1 and 16S for macro-fauna, and ITS for fungi and plants) and sequenced using in house Illumina NextSeq 500 and MiSeq platforms as previously described (reviewed in Creer et al. [26]).

***Bioinformatics analyses:*** Depending on available databases and other information, metabarcoding reads will be denoised and annotated against the reference database. Raw data sequence reads will be evaluated and processed by use of the *Quantitative Insights into Microbial Ecology* (QIIME, version 2) toolkit [27] and the USEARCH software [28-30]. Reads of worse quality (mean quality < 20) and sequences which contain ambiguous 'N' and homo-polymers will be discarded. Removal of chimeras will be conducted by use of UCHIME [30]. Taxonomy annotation will be assigned using usearch_global/usearch_local alignment or RDP_classification [31] or the lowest common ancestor (LCA) algorithm [32] against the SILVA for bacteria [33], Protist Ribosomal Reference database for algae and protozoa [34], and animal COI1 Classifier database (V4-ref) for macro-fauna [35].

Measures of alpha- and beta-diversity will be calculated to assess overall differences among species richness, abundance and structures of multiple communities. Alpha-diversity summarizes diversity of organisms in each sample, whereas beta-diversity measures differences of community structures among samples. Shannon entropy, an alpha-diversity measurement of richness and evenness, will be calculated at equal-depth. Beta-diversity, unweighted and weighted unifrac distances [36], between samples will be computed from feature tables. Methods will be sufficiently similar among locations so that comparisons can be made and to make the data of maximum utility to collaborators. Statistical analysis of taxonomic abundance and correlations with other environmental metadata will be performed using PRIMER7 with PERMANOVA+ add-on software (PRIMER-E Ltd, Ivybridge, UK) [37] and in R (https://cran.r-project.org/) with packages including VEGAN [38], PhyloSeq [39], metagenomSeq and APE [40]. Data analysis will be performed using Compute Canada high-performance computing systems as well as a cluster of 4 high-performance workstations available at the Doxey Lab and a supercomputer dedicated to genomic analyses available in the Giesy lab.

**Progress towards final products/outcomes during Phase 1, including major achievements.** Overall project goals included improved biomonitoring/ bio-assessment approaches through incorporation of next-generation tools and improved environmental management practices and policies through application of these technologies and transfer of knowledge to diverse stakeholder groups. Specific objectives were to: **1)**

**Develop a novel approach, based on eDNA, to assess absolute and relative changes in populations and communities in aquatic environments of northern and urbanized Canadian watersheds, and 2) Characterize communities in surface waters and sediments as indicators of ecosystem health under varying degrees of natural and anthropogenic stressors.** Much of the effort during Phase 1, which has focused on development of methods, has been completed and places the team in a strong position to continue with the other objectives of the study during Phase 2 of the 7-year program to assess absolute and relative changes in populations and communities in aquatic environments of northern and urbanized Canadian watersheds. We have conducted parallel, linked processes of technology development (left side of Fig. 1). Initially, we developed and applied a questionnaire for potential end-users to inform the development of the eDNA toolbox described here, and results of which have been collated and evaluated. By engaging end-users early and integrating them into our team we were able to ensure knowledge mobilization was achieved through co-creation of information. All aspects of the project are on schedule as proposed in the inception report. The new sequencing equipment has been purchased and installed, and methods have been developed and validated. All sub-projects in the original proposal have been initiated with our collaborators and we have actively engaged with our partners and completed some demonstration projects and knowledge transfers. Outputs include 1 report written and issued to a collaborator, 2 MSc theses, 11 peer-reviewed papers published or in press, 14 manuscripts in preparation and 44 abstracts read at regional, national and international meetings. In all cases, we have been able to leverage our resources by partnering with our collaborators. We have developed a web page to which we post progress reports and newsletters to collaborators. ***Objective 1.*** 44 SOPs were developed (available at https://gwf.usask.ca/edna/resources/standard-operating-procedures.php), validated, reviewed, and approved and are actively being used and available to all collaborators and other GWF projects. SOPs developed for eDNA cover field sampling, extraction of eDNA from multiple environmental matrices, qPCR of selected species including invasive and endangered species, PCR amplification of multiple barcode genes, next-generation sequencing and bioinformatics pipelines for characterizing bacteria, algae, protozoa and macro animal communities in aquatic ecosystems. The eDNA platform developed can be applied, modified, and optimized for various ecosystems. Objective 2 had multiple sub-objectives. ***Objective 2.1. Establish eDNA libraries for selected Canadian aquatic species and environments.*** A variety of freshwater fishes, zooplankton, and benthos were selected by end-users as critical species of concern in freshwater ecosystems of interest to them. Hence, we have developed or are in the process of developing databases for each of these taxonomic groups. Customized barcode databases of local freshwater fishes were developed for the eDNA toolbox. This database contains 226 mitochondrial, 12s rRNA sequences for 196 fishes, 265 mitochondrial, 16s rRNA sequences of 207 fish species, 312 mitochondrial cytochrome c oxidase I [*COI*] gene sequences for 245 fishes and 394 mitochondrial Cytochrome b [*CytB*] gene sequences of 252 fishes. An eDNA meta-barcoding primer set that detects local fishes and amphibians in the Grand River watershed has been developed using *in silico* methodologies. This eDNA metabarcoding tool is currently in the final stages of *in situ* validation by testing predicted performance of eDNA with field-collected specimens and voucher specimens obtained from the Royal Ontario Museum. Important quality control workflows have been established and validated to assess and mitigate potential presence of inhibitory substances as well as assess integrity of genetic material in samples of eDNA. Bioinformatics pipelines and classifiers are currently being optimized to identify fish and amphibian species from eDNA-NGS (metabarcoding) data. A database was developed for zooplankton in boreal lakes at IISD-ELA. About 80 molecular Operational Taxonomic Units (OTUs) were recovered from 18s rRNA-metabarcoding. Barcoding of zooplankton isolated and identified from IISD-ELA is underway. The final version of the zooplankton database will be constructed by compiling metabarcoding and barcoding data generated during the project. A barcoding database of freshwater macrobenthos was also initiated during Phase 1 and will continue during Phase 2. This database is being constructed based on communities in boreal lakes in collaboration with ORANO and the BOREAL project and seasonal wetlands of South Saskatchewan. eDNA libraries for algae, fish,

zooplankton, and macrobenthos communities, representing boreal rivers and lakes in cold regions of Canada, are being constructed based on samples sequenced among sub-projects. Additional eDNA libraries of selected environments, representing seasonal wetlands or irrigation watersheds in Saskatchewan and Alberta, are being constructed and sequenced. eDNA libraries of these selected environments will be used to assess status and trends of populations in aquatic ecosystems. ***Objective 2.2. Establish rates and reliability of detection of eDNA for key species defined by end-users: Lab and mesocosms calibration of eDNA.*** Several experiments were conducted under laboratory conditions to determine the reliability of eDNA detection (e.g. in water samples) of brook trout with closely related species present at different densities. Brook trout could be reliably detected in large tanks, without interference, and the quantitative polymerase chain reaction (qPCR) signal was proportional to numbers of fish in the tank. Considerable method development was required to verify methods and protocols, including types of filters, volumes, extraction and clean-up protocols required to ensure consistent results. "Foreign" (zebrafish) DNA was used to spike environmental samples and subsequently determine whether qPCR inhibitors were present. In controlled field studies, eDNA consistently detected wild trout placed in containers (1 h) and responses were density-dependent. When the stream bottom was disturbed strong inhibition was observed. When fish were removed from containers, eDNA rapidly declined over several days; however, samples were stable once frozen on filters. Several manuscripts are currently in preparation. ***Objective 3. Validate eDNA methodologies by comparing results to conventional taxonomic and ecological methods.*** This has been done by characterizing communities in surface waters and sediments as indicators of ecosystem health under varying degrees of natural and anthropogenic stressors. In 2017 we leveraged two studies that examined effects of anthropogenic stress. The first was a study of the Husky Oil Spill on the North Saskatchewan River (with Dept. Fisheries and Oceans). The second was a study of agricultural influences on communities of fishes in the Beaver River watershed (with Environmental Damages Fund). eDNA using barcoding of fishes was compared with results based on traditional fish monitoring techniques. For the Husky Oil Spill project, the overall equivalency between eDNA metabarcoding and netting of fish monitoring is about 85%, while the overall equivalency between eDNA metabarcoding and electrofishing in the Beaver River watershed was 72%. As part of the Husky Oil Spill project, a second-round validation and benchmarking of metabarcoding of fishes was conducted in August 2018 with 30 water samples per location. This validation of metabarcoding of fishes, also serves to benchmark fish metabarcoding for larger river ecosystems. All of the water samples of the 2018 campaign have been collected and processed. A manuscript that compares these results is currently being developed. Another project is underway to validate eDNA metabarcoding of zooplankton. This project leveraged three mesocosm studies conducted at the IISD-ELA: the first one entitled "Boreal lake Oil Release Experiment by Additions to Limnocorrals" (the BOREAL project); the second one entitled "Ecological Risk Assessment of Selenium" (ERASe project)"; and the last one entitled "Freshwater Oil Spill Remediation Study at the IISD-Experimental Lakes Area" (the FOReSt Project funded by NSERC). From June to August 2018, Zooplankton samples were collected from mesocosms of the BOREAL project and ERASe project to which various concentrations of diluted bitumen (Dilbit) or selenium had been added. We have completed the eDNA sequencing and are waiting for data on numbers and types of taxa present derived through traditional taxomic identification approaches as well as concentrations of the residues. Once that information is available, we will be able to compare the results of eDNA with traditional measures of species present as well as ecosystem-level parameters. A collaboration with the ongoing FOReSt Project (a similar study to determine effects and types of remediation of a spill of dilbit along shorelines of boreal lakes) will involve running a second round validation of zooplankton metabarcoding at IISD-ELA in June and July 2019. Two years ago we entered into a partnership to test the hypothesis that eDNA could be used to monitor for compliance in industrial effluents. Specifically, we started a monitoring program with Orano Canada Inc. (formerly Areva) to monitor downstream of their treatment plant. We collected eDNA samples in Spring and Fall of 2018 and consultants for Orano have collected parallel monitoring data for fishes and benthic invertebrates. eDNA results are currently being

benchmarked against historical data from Orano and the final validation will be conducted once we have the most recent results from the consultants conducting the traditional taxonomic surveys. We will prepare a joint publication with Orano. As part of the calibration studies, we are partnering with Alberta Environment and Parks. It has taken two years to obtain the necessary study plans and approvals in place. During that time the government in Alberta changed and we have shifted the program to Alberta Agriculture and Forestry (AAF), which is responsible for a monitoring program focused on surface waters used for irrigation. As part of that mandate, they are collecting samples for various physical/ chemical parameters and are conducting surveys of phytoplankton communities. In 2019, we collected 420 water samples in June, July, and August, to cover the blooming window of algae communities. eDNA from these samples is currently being sequenced. In the future we plan to collect eDNA synoptically with AAF from six locations per year. As data on chemical constituents and phytoplankton communities become available, results of eDNA barcoding and metabarcoding will be compared with the more traditional values as determined by microscopic examination. Initially this will focus on phytoplankton but eventually include fishes and invertebrates. This will allow for a test of the accuracy and precision for the more rapid eDNA approaches while calibrating them for comparison to historical data. We have signed an MOU to continue this project, in various watersheds until 2023. Several manuscripts are currently in preparation. **Objective *2.3a. Variability of eDNA in an instrumented watershed.*** Targeted eDNA barcoding, was applied in Washington Creek (Grand River sub-watershed), to monitor temporal and spatial changes in the population of resident brook trout (*Salvelinus fontinalis*). Although Washington Creek is a cold, groundwater-fed headwater stream it is located in an area that is affected by intense agricultural activities and urbanization that put pressure on the habitat of brook trout. Stream water sampling (3 × 1 L/site) for eDNA was combined with electrofishing conducted on a monthly basis (April to December 2018), at three sites (3 × 100 m) in Washington Creek. Hydrology and physical parameters (i.e., flow, temperature) were also recorded. Greater brook trout abundance and biomass in the summer months correspond to significantly greater detection of brook trout eDNA. With our partner, GRCA, spatial fish electrofishing surveys in Blair and Cedar Creeks (Grand River watershed) were also conducted, and also found a weak relationship between eDNA response and trout abundance (May-August). In collaboration with the Melimoyu Ecosystem Research Foundation (MERI, Chile) the distribution of invasive brown trout was successfully detected in remote streams of Chilean Patagonia (Melimoyu Nature Reserve). Additional controlled experiments are currently being conducted with specific DNA sequences introduced into a stream (Alder Creek) to examine their fates and persistence. A manuscript on factors affecting eDNA detection of brook trout is in final stages of preparation. **Objective *2.3b. Variability of eDNA in lotic and lentic habitats of Great Plains rivers.*** This objective is being addressed in multiple rivers and lakes in Saskatchewan, and Alberta. This descriptive data is not scheduled to be completed until the end of Phase 2. Geographic distribution patterns and temporal changes of eDNA-based aquatic microbial, invertebrate and vertebrate communities will be analyzed based on data collected during ongoing monitoring activities. **Objective 2.4. *Determine feasibility of including eDNA in existing biomonitoring and bio-assessment programs.*** This objective was not scheduled to be completed until full data sets are available during Phase 2. Specifically, interviews were completed in 2019 with approximately 40 collaborators and other water management professionals across Canada with extensive expertise in the legal, implementation, policy, practicality, and logistical aspects of incorporating eDNA technology into existing biomonitoring and bio assessment programs. The US M.Sc. student who conducted interviews visited the research group at Waterloo for several weeks during the fall of 2018 to work with them and to interview end-users with whom they partner. Results of this analysis of opportunities and constraints is currently underway, to be completed by Winter 2020. **Objective 2.5. *Develop technologies to solve pressing problems.*** We have been developing specific projects to meet the needs of our partners in government and industry. It was determined early on that application of eDNA approaches could be useful in doing surveys of endangered or invasive species – a mandate of provincial governments, a regulatory requirement of industry, and of great interest to conservation organizations. **Objective 2.*5a.***

***Detect invasive and threatened species.*** Over the previous two years, we have partnered with the Government of Saskatchewan and the Water Security Agency, and developed and optimized a rapid qPCR-based eDNA screening approach for zebra mussels, quagga mussels, and Prussian carp. We have screened over 120 sites where eDNA was measured synoptically with traditional collections and identifications of zebra mussels in lakes and reservoirs in Saskatchewan and Manitoba. The eDNA technique successfully identified the presence of mussels in Lake Winnipeg, where they have been established. In Saskatchewan, more than 100 sites were screened with no positive detections to date. Profs. Tim Jardine and Markus Hecker sit on the Province's Invasive Species Task Force, feeding this research directly into its zebra mussel monitoring program. Additional screening will be done in partnership with Saskatchewan Environment during Phase 2 in order to refine the monitoring program's sampling plan including analysis timing for quicker results and potential response. A study of wood frogs in Prairie wetlands revealed that eDNA was more effective at detecting organisms than conventional surveys (visual encounter). Future studies to be conducted under Phase 2 will involve development of targeted eDNA methods for additional species such as lake sturgeon. **Objective *5b. Assess utility of eDNA for biomonitoring in multi-use watersheds.*** This objective is ongoing in the North Saskatchewan River, the irrigation districts of Alberta, and the Grand River. Rates of detection by use of eDNA metabarcoding were compared with 3-pass electrofishing in Bauman Creek (rare Charitable Research Reserve), a tributary of the Grand River. Surveys were conducted along 4 reaches in three different seasons (summer, fall and spring). Sample processing is ongoing. In this same watershed eDNA metabarcoding was applied to characterise the amphibian species present in multiple vernal pools over a temporal and spatial scale from April – June and compared to conventional amphibian surveys (visual encounters, acoustic surveys). Environmental RNA (eRNA) was explored as a sensitive biomarker of stress that could be used in combination with eDNA. A pilot experiment in which water samples above and below the Waterloo WWTP have been analyzed for eRNA, and found that 1) microRNA (miRNA) can be detected and quantified from natural waters, and 2) specific stress miRNA can be detected in areas exposed to wastewater effluent that have previously been demonstrated to impact the physiology of fishes.

***New Project Deliverables and Timelines for Phase 2 Including Knowledge Mobilization Plan***

***Deliverables. Year 1-*** During year one, we will continue working with our partners as key end-users (Table 1). We will continue to collect field and laboratory data while continuing eDNA analyses of samples collected during Phase I and analyze data and prepare reports and joint manuscripts with partners. We will continue to meet with key partners and make presentations at local, national and international meetings. We learned during Phase 1 that it is not productive to host technical meetings where all collaborators are invited as it is logistically challenging to schedule meetings that can be attended by all members of the research team and collaborators. Furthermore, the diverse interests of the various groups mean that it is hard to make one size fits all. We learned that the cooperators prefer small group meetings where we can focus on their specific needs and explain the results of projects focused on those needs. Also, collaborators often do not wish to have the data released to a wider audience until they have had a chance to share the results with their management or with various responsible parties and to vet the results with internal technical experts before preparing more widely disseminated reports or technical publications. For instance for Saskatchewan Environment, with whom we have been developing and applying methods to survey for threatened and endangered species in various environments and surveying for targeted invasive species of concern included attendance by a Co-I (Hecker and/ or Jardine) at a meeting held by the province of SK Rapid Response Team for Aquatic Invasive Species (chaired by Ron Hlasny from Sask Environment). This Rapid Response Team, composed of representatives from multiple agencies (SK Ministries of Environment, Parks, Agriculture, Economy, and Highways, SK Water Security Agency, SaskWater, SaskPower) will act as an important boundary organization because it is tasked with preventing the entry and establishment of Aquatic Invasive Species. This team represents varied interests unified around a common goal, and it is seeking the

tools to do so. We have Memoranda of Understanding with Saskatchewan Environment and AEP and AAF and will hold annual meetings with those teams to present results and discuss plans for the following field season. It is far more efficient for key research faculty, post docs and students to travel to the collaborator. In that way we can minimize travel costs and maximize participation of staff working for the collaborators. The end users in the Grand River watershed will continue to be engaged through the GR Water Managers Committee as well as GR Recreational Fisheries Management Committee in which diverse partners are involved (federal, provincial, municipal governments, First Nations and NGOs) and Servos is a member. A draft document identifying diversity of end-user needs, common needs and applications, and format in which information is most useful for supporting needs / decisions (varying by end user type) will be developed. All of the PDFs, Ph.D. and M.Sc. students were recruited during Phase 1, have completed at least one year of their proposed 5-year programs of study, and are now in place and hitting the most productive parts of their tenure. The program has includedone PDF at UW and one at US and a total of five PhDs (3 at US and 2 at UW). We will continue projects started with collaborators during Phase 1 and initiate new sub-projects with continuing and new collaborators. We will continue collecting samples in coordination with provincial cooperators in Alberta and Saskatchewan so results of eDNA analyses can be compared with the results of on-going programs to monitor benthic invertebrates and fishes. We will also continue to collect samples for comparison with traditional taxonomic methods for the controlled mesocosm studies of selenium (Se) and diluted bitumen (dilbit) being conducted at the ELA. We will continue analyzing samples collected during Phase 1 and work that data through the analysis pipelines developed during Phase 1 and work with collaborators to prepare reports and manuscripts.

***Year 2-*** To expand the number of locations and types of environments studied, additional samples will be collected from identified study sites in consultation with end users for both conventional and novel approaches (e.g. eDNA, NGS), which we have found in the past to be critical boundary locations in developing a shared understanding of the local environment. eDNA methods will be validated by comparisons with field collections based on more traditional sampling analyses. In addition, methods for targeted species and communities (e.g. rare, endangered and invasive species) will be developed and optimized during Year 2. Field samples will be collected from local watersheds (Grand River, McKenzie Creek, Alder Creek), and locations in Saskatchewan and Alberta to be designated by provincial collaborators. This includes joint field experiences (PIs, students and end-users), where we can liaise with users to discuss application of eDNA tools in monitoring and assessment programs and train personnel in the methods for collection and shipping. A document identifying potential for application and integration of these tools in monitoring and assessment, including identification of potential technical, policy or regulatory barriers will be developed. We will present concepts and initial results at the Partners for the Saskatchewan River Basin meeting (October 2021). We will also continue the studies of inorganic and organic stressors in mesocosms in the ELA and will continue to collect samples in concert with our provincial cooperators in Saskatchewan and Alberta during routine monitoring programs. We will also continue to collect samples for comparison with results of surveillance programs to establish status of trends to monitor for effects of long-term changes in climate.

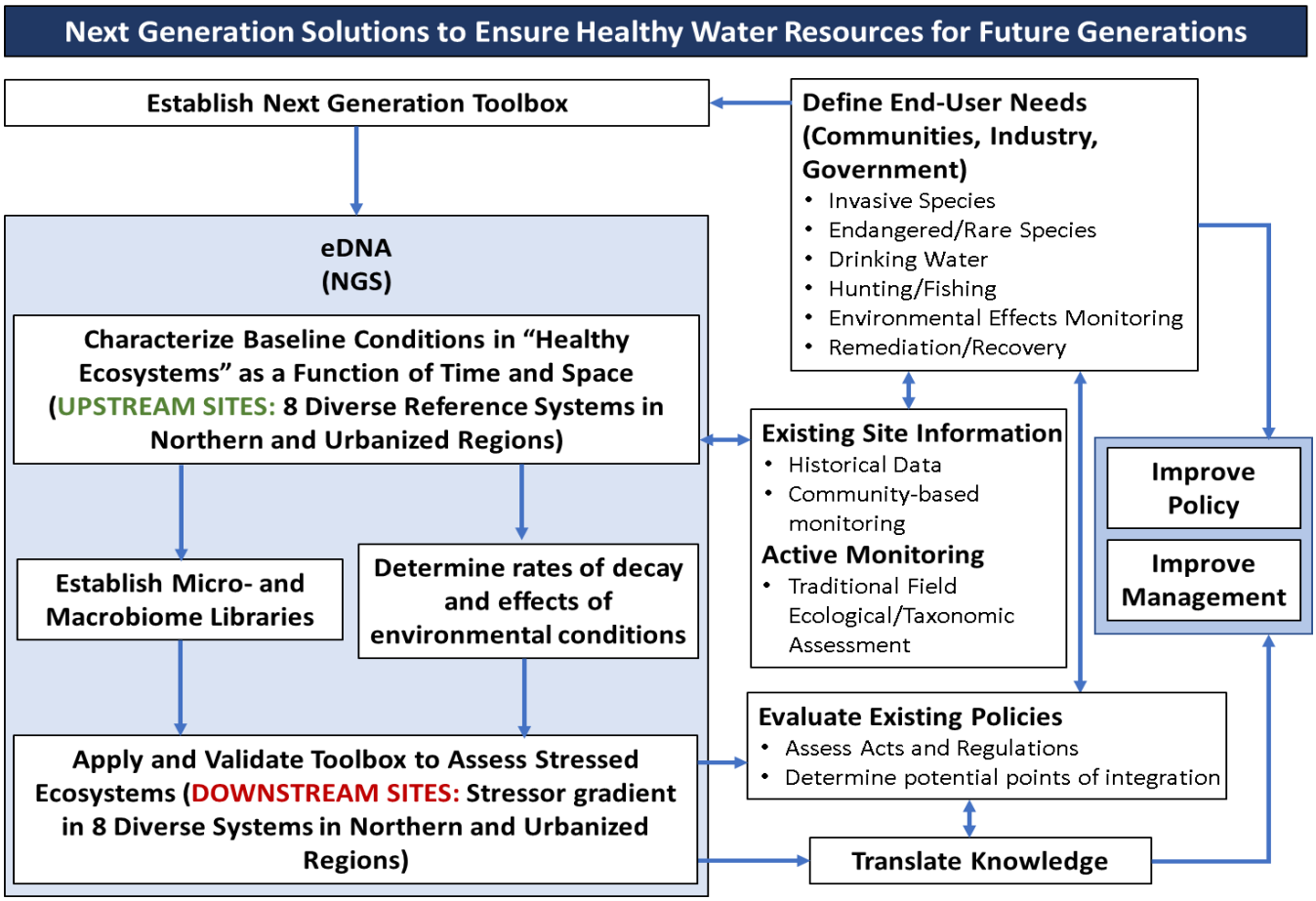
***Year 3-*** eDNA analysis will be applied to samples collected in targeted environments including diverse habitats and stressor gradients. Comparison of emerging eDNA approaches to conventional analyses will be made, and NGS data will be directly linked with water quality data across a gradient of anthropogenic disturbance. We will then develop a long-term strategy and identify immediate testing needs together with users, and conduct further validation of eDNA collection and assessment in partnered institution river systems.

***Year 4-*** Individual annual workshops and meetings will be held with each collaborator and partner agencies in SK and ON to deliver results, obtain feedback and set priorities for the future. During year 4, we will also be archiving all data in accessible platforms, completing data analyses and preparing reports and manuscripts and making presentations at meetings.

***Long-Term Deliverables during Phase 2***

***Years 1-4*** Methods developed during Phase 1 will be applied to sites and issues across the GWF platform. We will expand and leverage biological community libraries for diverse aquatic ecosystems to test hypotheses and address emerging end user needs. We will further explore temporal (seasonal and multi-year) and spatial patterns of biological communities (joint sampling program with end users). Also, a computing platform for the *in silico* 'archiving' of water samples and an interface for the interrogation of these archived samples will be established during years 4-7. The archive will include eDNA data for all samples collected and an analysis platform will be developed for integrated interrogation of these data resources. New partnerships have been formed with additional users to compare data acquired from next-generation technologies with those acquired by formal monitoring programs. We will continue to engage with national and international networks for application of approaches and will prepare papers on next generation monitoring strategies (e.g. advantages, limitations and potential applications) to assess aquatic ecosystem health (co-authored by investigators, collaborators and users). In partnership with the Computer Science Team, user-friendly interfaces will be developed that allow users to access a searchable database for eco-genomic data.

**Figure 1. Overall project schematic illustrating the parallel processes of technology development (eDNA) and evaluation of user-needs.**



A network of partners and collaborators are in place to support this project, including collaboration across all levels of government, industry, and public organizations (Table 1). These users will take part in workshops in years 1 and 3, hosted by US and UW. Workshops in year 1 will allow users to more clearly articulate the strengths and limitations of their current biomonitoring and bio-assessment approaches, which will inform the scoping and refinement of objectives in the technology development. Workshops in year 3 will allow end users to review results, provide feedback and identify new priorities.

US will be partnering with provincial agencies in Saskatchewan and Alberta. These agencies will provide support in many forms. We are in on-going discussing with them as to where to conduct specific sampling to best support their on-going monitoring efforts. Collaborators are also providing detailed chemical and physical data for environments assessed as well as providing detailed, quantitative results of biomonitoring programs involving benthic invertebrates, phytoplankton, zooplankton and vertebrates such as fishes**.**

**Roles of Collaborators and User/ Stakeholder Organisations**

In addition to the collaborative and joint research activities discussed in the previous sections, interactions with end-users will occur through their existing annual meetings. In the Grand River, we will present findings to the Grand River Recreational Fisheries Committee and the Grand River Water Managers Committee. In Saskatchewan and Alberta, we will present results to Partners for the Saskatchewan River Basin, the Delta Stewardship Committee (Cumberland House), and the Aquatic Invasive Species Task Force. A new partner, Matt Tyree (Sask Environment) will be involved with at-risk species detection by assisting with study design and field work coordination. Our international collaborator, Prof. Xiaowei Zhang of Nanjing University, will be participating in all aspects of developing methods for sequencing and subsequent statistical and informatics analyses of data. Prof. Giesy is currently working with Prof. Zhang and his post docs and students on projects developing and validating DNA barcoding techniques in China and assessing effects of stressors on prokaryotes and eukaryotes. Dr. Yuewei Xie, who completed his degree at Nanjing University under the direction of Prof. Zhang, is currently serving as the project manager (**US PDF 1)**. Last year he returned to China for a month to coordinate activities between the teams in Canada and those in Nanjing. He will continue to serve as the bridge to import technologies and to communicate with the team in Nanjing. It is anticipated that he will make several trips to Nanjing to coordinate efforts. In addition to our extensive international network of potential collaborators and partners that can contribute directly, we also work together with the core GWF universities with potential links to other GWF projects. Dr. Vince Palace of the Experimental Lakes Area (ELA) will be coordinating efforts between this project and on-going projects being conducted at ELA. Highly trained researchers have been conducting taxonomic surveys in lakes and streams of the ELA for 50 years. Several semi-controlled mesocosm studies with chemical stressors including inorganic selenium and organic constituents of dilbit are being conducted in which we are participating. Prof Klara Hilscherova of RECETOX, Masaryk University, Brno, Czech Republic is running a large TEAM project to develop approaches to monitor for biodiversity in European Rivers. Prof. Giesy helped design the program and will serve as the chair of the external advisory panel for this EU-Funded project. Prof. Hilscherova visited U of S last summer to learn techniques for collecting and analyzing eDNA and will continue to coordinate efforts in Europe to compare eDNA with results of monitoring of benthic invertebrates and fishes by use of traditional techniques for collecting and identification based taxonomy that uses morphology.

**Project Management**

The PI, J.P. Giesy will have overall responsibility for program oversight and coordination of reporting. We have organized the program into two nodes, based on geographic regions and locations of the researchers at the US and UW. The team at UW is headed by Prof Servos. The PI solicits sub-sections of annual reports, including progress on each objective and accumulates a compilation of meetings with collaborators,

abstracts read at meetings, reports issued to collaborators, and manuscripts published. In addition to communicating lists of outputs the PI, in consultation with Co-Is, compiles lists of outputs and significant outcomes from the ongoing research including interviews and or articles in the lay media. The PI, in consultation with the Co-Is, has developed a set of slides describing the experimental design, photos of environments, methods and procedures and annual outcomes and milestones that are provided to the central management team of the GWF. These slides can be shared with any other groups funded by the GWF or various collaborators. The PI will be responsible for ensuring the Knowledge Mobilization Strategy is unfolding as planned or adapted as necessary. While individual faculty members will be assigned to supervise PDFs and graduate students at their respective institutions, all hands conference calls (i.e. video conferencing) will be held regularly. During Phase 1 we held five of these video conferences and had two face-to-face meetings (one at US and one at UW). The team uses opportunities, such as national meetings, to facilitate all-hands meetings of the attending Co-Is and partners (e.g. Society of Environmental Toxicology and Chemistry, Canadian Ecotoxicology Workshop). Before initiation of each program element, detailed study plans have been and will continue to be developed. Detailed standard operating procedures (44 SOPs) including sample collection, tracking, storage and preparation, sequencing, data analyses and archiving have been developed. The PI will be responsible for coordinating formal communications to collaborators and end users. At a minimum, annual written reports and a verbal presentation, including Q&A session, will be provided for each project involving a joint collaboration with partners/end users. All SOPs and reports will normally be made available to all collaborators. The PI and all Co-Is will participate in writing reports and manuscripts with the individual PDFs and students. Students and PDFs will normally be first authors of all anticipated publications. When joint projects are conducted with collaborating partners or other academic institutions, co-authorship will be offered as appropriate. Proposed authorships are specified in the study plans before studies are initiated. The PI will coordinate opportunities for Co-PIs, PDFs and students, opportunities to make presentations at collaborating institutions, agencies, or scientific meetings. The PI will be responsible for budgetary oversight of the project at US and the Co-I (Servos) will fulfill a similar role at UW. Oversight will include allocating funding for travel, large purchases, and hiring students and PDFs. The PI will maintain authority for financial allocations, coordination and reporting.

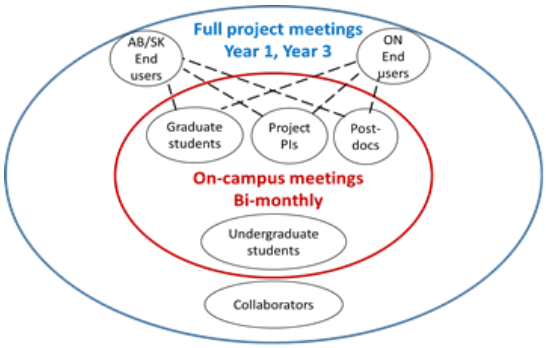



Fig. 2 Schematic illustrating planned interactions among the project team and end-users

**Data Management Plan:** Data management procedures have been developed and are being applied during the investigations to record, document, track, and compile investigative data into an overall project database. These detailed plans have been filed with the Data Management Core Team. To ensure generation of identifiable and usable data, organization and annotation of data is occurring prior to collection. Experiments and measures conducted/made as part of this project will conform to internationally accepted standards ensured through a range of activities as outlined in our sampling and analysis plans (SAPs) and SOPs. Furthermore, we will work via our Project Management scheme to ensure that all aspects of our project conform to the highest standards established for genomics. The project is expected to produce a range of types of data including genomics data, ecological survey data, water quality and chemical data, etc. as well as resources that help translate the data into knowledge and end user products, such as reports, factsheets, web user interface, etc. ***Recording of Data:*** Observations made and measurements taken during the studies will be recorded using appropriate hard copies in data sheets or logbooks as well as electronic formats. Data recorded in hard copy will be transcribed into electronic forms and proofed before use or integration with other data. Laboratory notebooks and field record sheets will be maintained in duplicate and archived

according to the specific SAP. All data manually entered or generated by instruments will be recorded into the laboratory information management system at each university after being reviewed (proofed) and approved by the responsible authority prior to being released. Chemical analysis, biological data, and associated QA/QC information will be entered into the project database (PDB), using SOPs, which will normally include: 1) Identification and tracking of samples and chain of custody; 2) Review and evaluation of analytical data against project-specific quality criteria; 3) data tables and where appropriate, 4) corresponding control charts/supporting information. ***Validation of Data:*** Validation of data is an integral part of the quality assurance program and consists of reviewing and assessing the quality of data and provides assurance that the data are of acceptable quality as reported. Data usability is the determination of whether or not a data set is sufficiently complete and of sufficient quality to be used in support of addressing study objectives. ***Analyses*:** Data analysis, including computation of summary statistics, standard errors and confidence intervals, will be conducted for this project. Genomics data will be processed by a range of bioinformatic applications such as DIAMOND, QIIME2 and USEARCH with web- and cloud-based database (SILVA, Genbank NR database, BOLD V4) as outlined in each SOP. . Raw sequences will be made available in the Federated Research Data Repository system (FRDR, www.frdr.ca/repo/) or other data bases, such as NCBI Sequence Read Archive. ***Data Storage and Retrieval:*** A database has been established for storage of original data, historical data, written documents, and data collected or generated during the experiments. All materials will be dated, carry the initials of persons responsible for the preparation of the document, and bear the project number. Access to the project files will be limited to those personnel assigned to this project. To ensure security of data, raw analytical data and associated metadata will be archived on secure University servers and backed up to the "cloud" by use of Federated Research Data Repository system (FRDR, www.frdr.ca/repo/). Meta data will include sample description, all analytical procedures performed, links to other associated GWF projects, analytical QA/QC data, and relationships to processed data files and subsequent 'downstream' files. Processed data files will also be archived in collaboration with the Data Management Core Team to provide data formatted for the GWF "Big Data' initiative. Archived data sets will be open to the public until research papers are accepted and ready for producing if there are no restrictions. Links or DOI numbers of publications should be provided in the archived data sets. If the data set has any access restrictions, restricted data sets are only accessible to relative partners. Information of embargo or restriction will be provided as well.

**HQP Training Plan**

This program will train several undergraduate assistants (4 part-time/y; 2 each at US and UW), 1 M.Sc. student (1 at UW) (Note: 1 M.E.S. student (US) has already been trained and will complete their degree program in the spring of 2020), 5 PhD students (3 at US, 2 at UW), and 2 PDF (1 each at US and at UW). HQP will acquire highly sought-after technical skills in field sampling, molecular biology, advanced genomics and bioinformatics data acquisition, and pipeline analysis. All Ph.D. students are already enrolled and working on their projects as part of the ongoing program. Furthermore, PDF and graduate trainees will be directly involved in interacting with end-users as part of their professional development (**Fig. 2**). The structure of the research program is such that it provides trainees with opportunities to develop end-user driven thought processes and development of "cutting edge" technologies for broad applications to emerging issues in aquatic ecosystem health. This unique training environment will result in HQP with a specialized set of skills for addressing emerging environmental issues from an end-user perspective, and make them attractive to employers in government, consulting, NGOs, and academia. In addition to preparing manuscripts and presentations for scientific peers, they will help prepare plain-language summaries of research findings to be delivered and presented to partner agencies, and assist in development of user-friendly visualization platforms/ interfaces. We have also allocated travel funds for students to take part in team meetings with end-users. Students will be part of interdisciplinary graduate programs at both US (Toxicology, SENS) and UW (Collaborative Water Program). The undergraduate students will be given

opportunities to participate in research activities and be included in on-campus meetings, to be held bi-monthly on the respective campuses, with phone access by rotating team members from the other campus. All HQP will interact with the faculty team that will act as co-supervisors and committee members. This team-oriented approach will also enable HQP to understand advantages and challenges of working in an interdisciplinary setting.

***Plan for spending existing budget August 31, 2020 and need for no-cost extensions.*** The budget provided for Phase 1 is being spent as specified in the original proposal. There have been no changes in how we proposed to spend the funds during Phase 1 and there will be no funds left at the end of Phase 1, and thus, there is no need to request a no-cost extension or give an explanation.

**Budget for Phase 2 with Justification (include cash and in-kind support from users/stakeholders, and defined allocations to Co-Is): Request from GWF for Phase 2 over 4 years=$1,267,476**

**Table 2. Summary of Budget request from GWF.**

| Activity/Year | 1 | 2 | 3 | 4 | Total |
|---|---|---|---|---|---|
| Personnel (HQP) | | | | | |
| PDF (2) (1 at US; partial at UW) | $49,670 | $58,612 | $61,210 | $69,274 | **$238,766** |
| PhD (5) (3 at US; 2 at UW) | $98,460 | $120,360 | $126,101 | $53,126 | **$398,047** |
| M.Sc. (1) UW | $10,850 | $18,600 | $19,158 | $8,222 | **$56,830** |
| Undergrad (4) 2 at US 2 at UW | $45,000 | $47,250 | $49,612 | $21,705 | **$163,567** |
| Technical staff (0.0) | | | | | |
| **Total Personnel** | **$203,980** | **$244,822** | **$256,081** | **$152,327** | **$857,210** |
| **Total Supplies/Services** | **$89,000** | **$97,000** | **$88,000** | **$41,666** | **$315,666** |
| **Travel** | | | | | |
| Field collections | $6,000 | $7,000 | $7,000 | $3,000 | **$23,000** |
| Coordination Meetings | $4,000 | $4,000 | $4,000 | $2,500 | **$14,500** |
| Scientific Conferences | $4,800 | $6,500 | $6,500 | $2,700 | **$20,500** |
| Knowledge Mobilization Workshops | $13,000 | $7,000 | $13,000 | $3,600 | **$36,600** |
| **Total Travel** | **$27,800** | **$24,500** | **$30,500** | **$11,800** | **$94,600** |
| **Total Requested from GWF** | **$320,780** | **$366,322** | **$374,581** | **$205,793** | **$1,267,476** |
| Other support-Cash | | | | | $154,415 |
| Other support-In kind | | | | | $1,269,350 |
| **Total other support** | | | | | **$1,423,765** |

1=Sept 2020-Mar 2021; 2= April 2021-Mar 2022; 3=April 2022-March 2023; 4=April 2023-Aug 2023

**Other Sources of Support:** ***Cash Support:***

Prof. Giesy will be dedicating part of his NSERC Discovery grant **($80,000**) and part of his US institutional support (**$24,415**) to support research in the area of eco-omics. Prof. M. Servos will contribute approximately **$50,000** of support over a three year period from NSERC Discovery and CRC. This project has leveraged funding provided by GWF for acquiring advanced instrumentation **($1,516,500)** by Thermo-Fischer Scientific for support and development of new hardware and software platforms for environmental analysis (**$416,500** based on 50% allocation of resources from the agreement with Thermo-Fisher to this project). Total cash funds, not including in kind, are **$154,415**, while in-kind support is **$1,269,350** for a leveraging of **$1,423,765** with a leveraging ratio of **1.12.** To stretch funding, we will be encouraging students

and PDFs to apply for federal stipend support and to apply for matching travel funds whenever possible. During Phase 1 one student received a Dean's scholarship, another received a departmental devolved scholarship and one a NSERC PGSD and two received RARE scholarships. Some of these continue into next year, but in Phase 2, the project will need to support these students through the end of their programs.

**Other Sources of Support:** ***In-Kind Support:*** At this point, we have secured in-kind support and access to samples from the identified research partners and, if successful, we will use GWF cash to leverage additional cash funding from provincial agencies. We will collaborate with agencies to gain access to sites and then collect synoptic samples so that the eDNA data can be compared to results of more traditional methods of sampling and identification and quantification. For example, benthic invertebrate assemblages are well-described along the South Saskatchewan, North Saskatchewan and Saskatchewan rivers [41]. Similarly, Orano, through their EEM requirements, has generated extensive data sets on fish and invertebrate communities in boreal lakes downstream of their mining operations, and have provided sampling support for the studies conducted under Phase 1 in the amount of approximately **$100,000** over the past years. We will capitalize on this background knowledge to pre-populate species databases for sites of interest, and then examine eDNA-based approaches at comparable locations. Studies in the central Grand River over the last decade have created a database on the ecosystems and tissue archive (funded by NSERC, CWN, etc.) that will be available. The ***Ministry of Natural Resources and Forestry*** and the Grand River Conservation Authority have agreed to provide access to data bases on fish distributions and water quality in the watershed. ***Trout Unlimited, Friends of the Grand River*** and ***Six Nations of the Grand River*** will provide local knowledge. All of these organizations have agreed to participate in meeting/workshops to exchange knowledge and provide advice. ***IISD-ELA*** will also provide access to their 48-year database on meteorology, hydrology, water chemistry, and the food webs of ELA lakes to provide a freshwater context for the eDNA studies proposed by Drs. Giesy and Servos (estimated value of >**$10,000**). ***IISD-ELA*** will also provide in-kind contributions of at least **$40,000** to the program, which includes time spent by Dr. Palace (4 wks/yr * $2800/wk * 3 yrs; total **$33,600**), IISD-ELA's Head Research Scientist, to attend planning meetings, analyze and assess data and contribute to manuscript writing and presentations of research results at conferences and community workshops. We will also contribute per diem expenses of IISD-ELA staff at the field station while working on the project **($16,000**). Please note that per diem costs for NSERC supported researchers included in the project are out-of-pocket expenses for ELA and cover the room and board of researchers at the facility.

***Alberta Department of Agriculture and Forestry*****:** Estimated in-kind support through personnel to develop study plans, provide archival data and to collect samples is **$75,000** over three (3) years during Phase 2. ***Saskatchewan Environment:*** Sample collection for at-risk species work in remote locations **($75,000)** and staff time in project design, meeting attendance and student supervision **($30,000)**. ***Nanjing University***: Prof. Giesy is a co-investigator on several projects funded to Prof Zhang by the Chinese Government. These funds cannot be expended in Canada, but will support activities to develop and validate eco-omic methods and support students from US when they are in China. These projects include: "Solutions for present and future emerging pollutants in land and water resources management." funded by European Commission, Directorate for Innovation and Research, Directorate I Environment, "Toxico-genomic Assessment of Emerging Environmental Pollutants Using Novel Functional Genomic and High Throughput Technologies", (***Total ¥350,000***) (**$26,250)**, "Development and application of high throughput toxicity test tools for chemical management" Grant for Innovation Talents of Jiangsu Province (***Total ¥1,000,000***) (**$75,000)**, and "Key technologies of ecological control and water quality improvement of the heavily polluted rivers in the Huai Riverine basin". National Water Pollution Control and Treatment Project of China (Project 2017ZX07602-002), (***Total ¥3,800,000***) (**$357,600)** for a total of **$458,850**. ***Grand River Conservation Authority:*** Will provide monitoring data and staff time estimated to have a value of **$10,000/yr** for a total of **$30,000**. ***Trout Unlimited Canada:*** Will provide assistance with collection of data

worth approximately **$3,000/yr** for three years for a total of **$9,000.** ***The Ontario Ministry of Natural Resources and Forestry:*** Will provide approximately **$4,500** worth of data collection support. ***The MERI Foundation*** will provide logistical support for sampling. Although not quantified this support is expected to be approximately $10,000 per year. ***The rare Charitable Research Foundation*** is providing an estimated $4,500 for staff time, data and site access. They also provide competitive student scholarships.
***Masaryk University (MU), Czech Republic*** is conducting several joint projects where students and faculty from US and MU have traveled to the other institution to collect samples and receive training and do instrumental analyses.

**GWF request**
***Highly Qualified Personnel:*** >50% of the GWF request is for HQP.
***Post-doctoral fellows***: Two (2) PDFs are needed to support overall project management, one at US and one at UW. At UW, the partial PDF (supervised by Servos and Doxey; supported by Craig and Katzenback) will take leadership of application of eDNA methods on the Grand River and its tributaries as well as controlled laboratory studies, QA/QC, and bioinformatics. The PDF at US in the area of ecogenomics (supervised by Giesy, supported by Jones, Hecker and Jardine) will be Dr. Yuwei Xie who will serve as project manager. The PDF at US has been unionized and base stipends have been increased, annual increases have been negotiated and there is a retroactive increase we have had to pay. We now also need to pay for the benefits for PDFs, so this has been included into the annual costs.
***PhD students:*** Five (5) Ph.D, three at US and two at UW will continue projects started during Phase 1. At US, one Ph.D. (Hecker, supervisor; Giesy and Jones, committee members) will focus on eDNA evaluation at ELA and one Ph.D. (Giesy and Jones, supervisors; Jardine, committee member) will evaluate linkages between chemical profiles in water and species assemblages across a gradient of anthropogenic stressors in the Peace, Athabasca and Saskatchewan rivers. This student will focus on assessing endangered and introduced species. The third Ph.D. student (supervised by Giesy and Jones) will be responsible for coordinating and conducting studies with AAF and AEP. One Ph.D. at UW (supervised by Katzenback and Craig with Doxey and Servos as committee members) will focus on lab/field calibration of fish and amphibians while the second (supervised by Servos and Craig with Doxey and Katzenbach as committee members) will focus on McKenzie Creek (Six Nations).
***M.Sc. and M.E.S. students***: Two sequential M.Sc. students at UW will be involved in the method development and fate studies on DNA in small creeks (supervisor Servos with support of others).
***Undergraduate students:*** Two BSc Students will be recruited from US to pursue term research projects over the academic year. At US they will assist with field and laboratory sub-projects arising from the studies of PhD students and PDFs, with one likely taking a role collaborating with Orano in EEM applications. The other BSc student will participate in laboratory studies and work on developing eDNA libraries. At UW undergraduate students are involved through the Work Study program, Coop and undergraduate theses. For all of the undergraduate students the goal is for them to have a positive experience doing research and to learn how to be a researcher to prepare for professional school. The projects will include everything from the conception and planning of independent studies to collecting and analyzing data and finally preparing manuscripts for publication and giving papers at regional and national conferences.
***Technical staff:*** There will be no technician supported by GWF funding to this project, but rather, Prof. Giesy supports Anne St. Ives and M. Servos supports Leslie Bragg, through their Chairs, that assist PDFs and students working on the project.
***Supplies and Services (4 year totals)***
***Equipment and Facilities:*** Most of the $15,016 over 4 years will be used for rental and operation of vehicles for travel to field sites. These costs are based on experience during Phase 1.
***Field supplies, sample collections and site characterization:*** A total of $38,500 will be needed for supplies to collect samples over the entire project in Phase 2. This includes: 1) materials for collection of eDNA***:***

filters, sample vials, filtration pumps, etc. *Total cost = $10,500*, 2) biochemical/ molecular supplies: for qPCR based targeted analyses of select species (invasive and threatened species). We will be supplementing this budget with funds from Prof. Giesy and Servos. *Total cost = $37,500*.
This estimate is based on costs expended for sampling supplies during Phase 1.
***DNA sequencing materials:*** Kits are needed for extraction, amplification and sequencing of DNA. This is by far the greatest cost for the Phase 2 projects at $7,200 per kit. We will be supplementing this with funds from Profs Giesy and Servos. *Total cost = $137,500*. Of this biochemical/ molecular supplies make up *$37,500* for qPCR based targeted analyses of select species (invasive and threatened species).
***Labware/ disposables:*** Day to day use of glassware, etc. Estimated based on past projects. *Total cost = $23,916 or $6,000/yr*.
***Computing and office supplies:*** We have purchased all of the students new computers during Phase 1 of the project from non GWF funds so are requesting no additional funding for computing. We have secured access to computing cores and cloud storage through Compute Canada.
***Publication costs:*** We have requested *$5,000* for cost of publication. This will offset a fraction of the cost for the 15 or so anticipated publications, so we will be subsidize with funds from all of the Co-Is.
***Travel plus costs at ELA***
***Field work:*** To obtain water samples, we will need to visit field sites at various times of the year, ideally from locations where conventional taxonomy is being done as part of larger projects or for annual monitoring by agencies or industry. The work in the Grand River will be leveraged with other grants that are related to biomonitoring and assessments being conducted. Resources from the Canada Research Chair in Water Quality Protection (Servos; $50K) will also support field collections and sample analysis. Instrumentation (e.g. LC-MS/MS, PCR, etc.) as well as field equipment (e.g. truck, trailers, boats and electro-fishing) will be made available through SOWC and the Biology Core facilities (e.g. sequencing, Wet Lab). We are requesting a small amount of field travel funds to enable collections from sites of interest that lack current sampling programs. Students and the Research Technician will spend approximately 18 days in the field. Because of differences in proximity to sample sites, we have budgeted a larger amount for AB/SK field work ($2,000 per trip vs $500 per trip). Total cost requested from GWF for travel is *$23,000*. Since travel for collections and per diems for staying at the ELA will be greater than this, approximately $46,000 cash will be contributed by the Co-Is from institutional CRC funds. In kind support from partners will also contribute to support travel.
***Coordination meetings, outreach and workshops:*** We have not requested travel funds for coordination meetings. These will be done by teleconference and where possible coordination meetings will be combined with travel to various scientific meetings. Most of the travel budget will be to support travel of researchers and or collaborators to transfer technology.
***Scientific conferences:*** Support for faculty and students to attend local, regional, national and international meetings is critical to their professional development. We are requesting a total of = *$20,500* for national or international meeting (e.g. CEW, SETAC). This will be supplemented by funds from the Co-Is.
***Knowledge Mobilization Workshops***: We anticipate full one-day workshops involving the entire project team in years 1 and 3, one each in SK and ON. For these meetings, we will provide a travel allowance for key partners to attend. We will also send key members of the team to the meeting hosted by the other university (i.e. 1 or 2 individuals from US will attend the UW meeting, and vice versa). Over the four years of the project, annual meetings will be held between individual sub-project research teams of faculty and students and collaborators. *Total cost = $36,600*.

**Linkage to GWF Core Support teams:** The group continues significant interactions with the *Knowledge Mobilization (KM) Team*. Stephanie Merrill, the US KM specialist has been well connected to our project. We worked together to assess the opportunities and roles of the KM team in supporting our research group through Phase 1 and with the KM and Communications teams to design and produce our project's logo.

Stephanie attends and gives input on KM opportunities at our regular team and bi-group meetings with the UW team, and has reviewed the website SOPs, and study plans for appropriate clarity and language. She has also worked closely with Renata Mont'Alverne, the M.E.S. student being supervised by Profs. Noble and Jardine, who continues to assemble information from end-users on opportunities and challenges of using eDNA in aquatic assessment and monitoring. Stephanie assisted Renata extensively in the preparation of her interviews, in providing names of potential participants, and reaching out to her own network of colleagues asking to participate in the study. We valued the opportunity for Yuwei Xie and Renata Mont'Alverne to present two posters (entitled "Opportunities and constraints in applications of eDNA techniques in environmental assessment of aquatic ecosystems" and "Next generation environmental DNA for biomonitoring of multiple communities") during the Agriculture-Water Research Expo on June 14, 2019, and Markus Hecker to present on our invasive species work at the upcoming Partners for the Saskatchewan River Basin AGM – opportunities which Stephanie has facilitated to make happen. She is currently working with Prof. Tim Jardine to document the knowledge mobilization impact from our invasive species monitoring in partnership with the SK Ministry of Environment for use as a GWF KM case study, future magazine publication and potential media opportunities. Stephanie is committed to continuing to work with our team into Phase 2. An important question that will be explored in years 3-7 is: Can these new bioassessment indicators be integrated into existing monitoring and assessment programs and will interpretations of the results be acceptable by our partners and potential other end-users? This requires an exploration of decision-making by regulators and proponents and requires appropriate communication about strengths and weaknesses relative to existing monitoring approaches. Integration of the tools developed during this project with existing end-user monitoring programs relies on the development of software that is end-user friendly. Stephanie will be engaged in helping to facilitate some of these discussions that seek feedback from end-users, and give input into software development. We have also worked with Nancy Goucher, Knowledge Mobilizations Specialist, and Allie Dusome, Communications Specialist at UW to prepare presentations.

Collaborating with the *Data Management Team*, we developed a Data Management Plan at the project level. This Data Management Plan is compatible with both the "Global Water Futures – Data Policy" (Revised March 4, 2019) and the science community standards of genomics data. We also helped the *Data Management Team* to develop and test inhouse methods for creating metadata being developed to label, curate and retrieve data. Supported by *Computer Science Team*, we formed a project-specific solution for data storage and data processing on Compute Canada.

Collaboration with the *Computer Science Team* will be required to develop computing systems and algorithms for integration of NGS data. Core technologies for testing and implementation of such a water futures toolbox have been established through the early investment of the GWF in state-of-the-art NGS capabilities. The *Computer Science Team* has been helpful in establishing the contacts with *Compute Canada* to get the necessary access to computing cores and data storage space. We will need additional support as monitoring data from monitoring studies in Saskatchewan and Alberta are assessed.

Our group will require continued support from the *Waterloo Technical Team* (Water Quality and Aquatic Ecosystem, Smart Watershed) for sample collection, observational analysis and data integration of eDNA and watershed data. Linkage of the "Big Data" collected from this program with other GWF proposals will require assistance of the *Core Modelling Team* to incorporate multi-parameter and assessment data tools in modeling the interaction(s) between changes in hydrology, water quality, and abundances of species to predict impacts of our changing world on aquatic ecosystem health on a pan-Canadian scale.

## References


1. *Shokralla, S., et al., Next-generation sequencing technologies for environmental DNA research. Mol Ecol, 2012. 21(8): p. 1794-805.*
2. *Sogin, M.L., et al., Microbial diversity in the deep sea and the underexplored "rare biosphere." Proc Natl Acad Sci U S A, 2006. 103(32): p. 12115-20.*
3. Saxena, G., et al., *Ecogenomics reveals metals and land-use pressures on microbial communities in the waterways of a megacity.* Environ Sci Technol, 2015. **49**(3): p. 1462-71.
4. Xie, Y., et al., *Using in situ bacterial communities to monitor contaminants in river sediments.* Environmental pollution, 2016. **212**: p. 348-357.
5. Amaral Zettler, L.A., et al., *Microbiology: eukaryotic diversity in Spain's River of Fire.* Nature, 2002. **417**(6885): p. 137.
6. Deiner, K., et al., *Environmental DNA reveals that rivers are conveyer belts of biodiversity information.* Nature communications, 2016. **7**: p. 12544.
7. Gibson, J., et al., *Simultaneous assessment of the macrobiome and microbiome in a bulk sample of tropical arthropods through DNA metasystematics.* Proc Natl Acad Sci U S A, 2014. **111**(22): p. 8007-12.
8. Thomsen, P.F. and E. Willerslev, *Environmental DNA–An emerging tool in conservation for monitoring past and present biodiversity.* Biological conservation, 2015. **183**: p. 4-18.
9. Feris, K.P., et al., *Hyporheic Microbial Community Development Is a Sensitive Indicator of Metal Contamination.* Environmental Science & Technology, 2009. **43**(16): p. 6158-6163.
10. Sabater, S., et al., *Monitoring the effect of chemicals on biological communities. The biofilm as an interface.* Analytical and bioanalytical chemistry, 2007. **387**(4): p. 1425-1434.
11. Yergeau, E., et al., *Next-generation sequencing of microbial communities in the Athabasca River and its tributaries in relation to oil sands mining activities*. Appl. Environ. Microbiol., 2012. **78**(21): p. 7626-7637.
12. Hajibabaei, M., et al., *A new way to contemplate Darwin's tangled bank: how DNA barcodes are reconnecting biodiversity science and biomonitoring.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2016. **371**(1702): p. 20150330.
13. Goldberg, C.S., et al., *Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders.* PloS one, 2011. **6**(7): p. e22746.
14. Maruyama, A., et al., *The release rate of environmental DNA from juvenile and adult fish.* PLoS One, 2014. **9**(12): p. e114639.
15. Pilliod, D.S., et al., *Factors influencing detection of eDNA from a stream-dwelling amphibian.* Molecular Ecology Resources, 2014. **14**(1): p. 109-116.
16. Barnes, M.A., et al., *Environmental conditions influence eDNA persistence in aquatic systems.* Environ. Sci. Technol, 2014. **48**(3): p. 1819-1827.
17. Eichmiller, J.J., S.a.E. Best, and P.W. Sorensen, *Effects of temperature and trophic state on degradation of environmental DNA in lake water.* Environmental science & technology, 2016. **50**(4): p. 1859-1867.
18. Jane, S.F., et al., *Distance, flow and PCR inhibition: e DNA dynamics in two headwater streams.* Molecular ecology resources, 2015. **15**(1): p. 216-227.
19. Strickler, K.M., A.K. Fremier, and C.S. Goldberg, *Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms.* Biological Conservation, 2015. **183**: p. 85-92.
20. Foppen, J.W., et al., *Using multiple artificial DNA tracers in hydrology.* Hydrological Processes, 2011. **25**(19): p. 3101-3106.
21. Foppen, J.W., et al., *Development of a methodology for the application of synthetic DNA in stream tracer injection experiments.* Water resources research, 2013. **49**(9): p. 5369-5380.

22. Liao, R., et al., *A DNA tracer system for hydrological environment investigations.* Environmental science & technology, 2018. **52**(4): p. 1695-1703.
23. Hegmann, G. and G.T. Yarranton, *Alchemy to reason: effective use of cumulative effects assessment in resource management.* Environmental impact assessment review, 2011. **31**(5): p. 484-490.
24. Jerde, C.L., et al., *"Sight-unseen" detection of rare aquatic species using environmental DNA.* Conservation Letters, 2011. **4**(2): p. 150-157.
25. Wilson, C., et al., *Environmental DNA (eDNA) monitoring and surveillance: field and laboratory standard operating procedures*. 2014.
26. Creer, S., et al., *The ecologist's field guide to sequence-based identification of biodiversity.* Methods in Ecology and Evolution, 2016. **7**(9): p. 1008-1018.
27. Bolyen, E., et al., *QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science*. 2018, PeerJ Preprints.
28. Edgar, R.C., *UPARSE: highly accurate OTU sequences from microbial amplicon reads.* Nature methods, 2013. **10**(10): p. 996.
29. Edgar, R.C., *UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing.* bioRxiv, 2016: p. 081257.
30. Edgar, R.C., *UCHIME2: improved chimera prediction for amplicon sequencing.* bioRxiv, 2016: p. 074252.
31. Wang, Q., et al., *Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy.* Appl. Environ. Microbiol., 2007. **73**(16): p. 5261-5267.
32. Huson, D.H., et al., *Integrative analysis of environmental sequences using MEGAN4.* Genome research, 2011. **21**(9): p. 1552-1560.
33. Quast, C., et al., *The SILVA ribosomal RNA gene database project: improved data processing and web-based tools.* Nucleic acids research, 2012. **41**(D1): p. D590-D596.
34. Guillou, L., et al., *The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy.* Nucleic acids research, 2012. **41**(D1): p. D597-D604.
35. Porter, T.M. and M. Hajibabaei, *Automated high throughput animal CO1 metabarcode classification.* Scientific reports, 2018. **8**(1): p. 4226.
36. Lozupone, C. and R. Knight, *UniFrac: a new phylogenetic method for comparing microbial communities.* Appl. Environ. Microbiol., 2005. **71**(12): p. 8228-8235.
37. Clarke, K. and R. Gorley, *Primer.* PRIMER-e, Plymouth, 2006.
38. Dixon, P., *VEGAN, a package of R functions for community ecology.* Journal of Vegetation Science, 2003. **14**(6): p. 927-930.
39. McMurdie, P.J. and S. Holmes, *phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data.* PloS one, 2013. **8**(4): p. e61217.
40. Paradis, E., J. Claude, and K. Strimmer, *APE: analyses of phylogenetics and evolution in R language.* Bioinformatics, 2004. **20**(2): p. 289-290.
41. Phillips, I.D., et al., *Thermal alteration and macroinvertebrate response below a large Northern Great Plains reservoir.* Journal of Great Lakes Research, 2015. **41**: p. 155-163.

# Curriculum Vitae

**Name/Affiliation:** Paul M. CRAIG, Ph.D., M.Sc., University of Waterloo

**Current Job Title:** Assistant Professor, Biology, 2014-Present

## Key Awards and Distinctions

- 2019 Ontario Early Researcher Award
- 2018 Outstanding Performance award, University of Waterloo
- 2014 President's Award from the Canadian Society of Zoologists
- 2010 NSERC Post Doctoral Fellowship
- 2006 NSERC Post Graduate Scholarship Doctoral Program
- 2005 Ontario Graduate Scholarship
- Published 35 peer-reviewed works, including 31 peer-reviewed open literature articles. Although an early career researcher, Dr. Craig is well cited with >1040 citations, a Hirsch (H) index of 16, and an i10 Index of 23 (Google Scholar®).
- Recognized as a leader in my field; currently serving on the Editorial Board for Comparative Biochemistry and Physiology, Frontiers in Physiology, and have acted as a reviewer for national granting agencies in Canada and Great Britain, and for a variety of comparative physiology and molecular journals.
- Leadership roles in regional and national societies, including Councillor for the Canadian Society of Zoologists. Currently serving as chair for the University of Waterloo Biology Outreach program and the Faculty of Science representative for the Bridges Lecture series. Recent (2017) co-organizer of a regional physiology and biochemistry workshop.
- Current vice chair of animal care committee at the University of Waterloo

## Research Funding

Current funding: (***Total $3,071,704***)

- Using biological tools to predict impacts of climate change and contaminants on aquatic health, Ontario Early Researcher Award, **$150,000**.
- Genetic and probiotic improvement of immunity in triploid Chinook salmon, NSERC Strategic Projects, **$556,000** (Co-Investigator, $101,502 my portion)
- Diagnosis of multiple stressors to adverse ecological responses across watersheds, Global Water Futures: PI: Mark Servos. 2018-2021, **$300,000** (Co-investigator, $100,000 my portion)
- Next generation solutions to ensure healthy water resources for future generations, Global Water Futures. PI: John Giesy. 2017-2020, **$1,391,215** (Co-Investigator, $99,972 my portion).
- Canadian Foundation for Innovation Internal Operating Funds, **$29,476**.
- Integrative responses of fish to environmental stressors, John Evan Leaders Fund – Canadian Foundation for Innovation (JELF-CFI) and Ontario Ministry of Research and Innovation Funds (ORF), 2015-2017, **$300,000** (Co-Investigator, $150,000 my portion).
- Environmental regulation of epigenetics in aquatic teleosts, NSERC Discovery Grant, 2015-2020, **$145,000.**
- Laboratory Start Up Funds, Dept. of Biology, University of Waterloo, 2014-2019, **$200,000**.

*Global Water Futures: Solutions to Water Threats in an Era of Global Change*

**Most Significant Contributions** (references[x] correspond to Key Publications)

**Multiple stressors and metabolism**[1,2,4,5,7-13,16,19]. While biomarkers are frequently employed to assess the sub lethal effects of contaminant exposure, there is a lack in knowledge regarding the mechanistic linkage between subcellular impacts relating to higher-order effects, which effectively limits the predictive power of useful biomarkers. An alternate approach to biomarker identification examines the overarching bioenergetics effects of stress using energetic biomarkers, such as metabolism and oxygen consumption, which compliment traditional molecular and phenotypic biomarkers currently employed. Dr. Craig has established himself as a researcher that focuses on the bioenergetics and energy expenditure attributed to multi-stressor exposure in aquatic species. Using both a molecular basis of mitochondrial respiration and cellular energy sensing, to whole animal oxygen consumption and metabolism, Dr. Craig has identified commonalities between exposure, wherein basal metabolic rate is significantly increased, in both controlled laboratory experiments and field collected species, which directly impacts overall performance and fecundity of species. This is a new approach to understanding multiple stressors, and places Dr. Craig research at the forefront of whole animal biomarker identification, which has significant potential with non-invasive assessment of multi-stressor exposure in aquatic species. **Epigenetics and multiple stressors**[3,6,7]. Phenotypic plasticity is a term that has gained a great deal of momentum recently, however the factors that regulate phenotypic plasticity are often overlooked, namely epigenetic factors. Epigenetics has been at the forefront of gene transcription and translation regulation for the past 10 years in biomedical fields, and only recently have non-model aquatic species been studied. microRNAs (miRNA) are epigenetic factors that regulate the translation of mRNA into protein, essentially dictating the phenotypic response in an organism. Dr. Craig's recent research has focused on the miRNA patterns associated with exposure to multiple stressors, with a particular focus on antidepressants found in wastewater effluent. Evidence from this line of study has indicated sensitive miRNA biomarkers are consistent with antidepressant exposure across species (zebrafish, goldfish, trout). **Epigenetics and metabolism**[6,7,11,14,15]. Dr. Craig's research has advanced our understanding of dietary methionine restriction and epigenetic machinery. At the time of these experiments, dietary MR was promoted in biomedical research as a means to decrease reactive oxygen species production, increase longevity, and promote altered energy expenditure in human subjects with metabolic syndrome through fat oxidation, although the mechanisms involved were unclear. Using trout as a model for glucose intolerance was key in determining the mechanisms of dietary MR. Furthermore, these studies established the first steps in determining dietary regulation of epigenetics, namely the methylation of DNA. Furthermore, Dr. Craig was able to develop a non-radioactive method to use cell culture to validate the response that Dr. Craig found in a whole animal model. This line of research was critical in determining the mechanisms that are involved in MR but also provided the first evidence in aquatic species that diet plays a significant role in DNA methylation. **Genomic endpoints of chronic copper exposure**[17,18,20]. Dr. Craig's initial research was the first to examine chronic impact of low dose, waterborne copper exposure in zebrafish and its genome-wide effects. This was essential in identifying the gene endpoints of chronic copper exposure across various water chemistries, which is essential for the incorporation of genomic data into a biotic ligand model for the prediction of chronic copper toxicity in a tropical model species. Using a molecular response was key in establishing distinct transcriptional events that are associated with decreased growth, impaired ion homeostasis, endocrine disruption, and decreased fecundity. Dr. Craig's approach has been repeated for numerous studies examining a variety of different contaminates, and many have now recognized the importance of mixed water chemistry and multiple environmental stressors to test more natural conditions. These were seminal papers in advancing the understanding of genomic endpoints related to copper toxicity.

## Relationship to Other Grants

I have secured funding for acquisition of cutting-edge research infrastructure and thus there are no available funds to cover the operational costs associated with the current proposed research.

2) **Title:** Genetic and probiotic improvement of immunity in triploid Chinook salmon. **Funding Source:** NSERC Strategic Projects 2018-2021. **PI:** Brian Dixon**. Amount:** $556,000. **Relationship to Current Proposal:** This project examines the epigenetic mechanims that drive adverse immune function in farmed, triploid Chinook salmon using next generation sequencing technology. While there is methodological overlap in sequencing techniques, there is no budgetary overlap associated with this proposal.

**2) Title:** Using biological tools to predict impacts of climate change and contaminants on aquatic health. **Funding Source:** Ontario Early Research Award 2019-2024**. PI:** Paul Craig. **Amount:** $150,000. **Relationship to Current Proposal:** This fund is directed towards supporting HQP affiliated with my NSERC Discovery grant, and has no budgetary overlap with this proposal.

**1) Title:** Diagnosis of multiple stressors to adverse ecological responses across watersheds. **PI:** Mark Servos. **Amount:** *$300,000*. **Relationship to Current Proposal:** The research associated with this grant focuses on the development of metabolic and alternate physiological endpoints of wastewater effluent exposure in darter species in the Grand River. This uses respirometry and other physiological measurements to incorporate into the development of useable model for effluent contaminant regulation. There is no budgetary or methodological overlap associated with this proposal.

**3) Title:** Environmental regulation of epigenetics in aquatic teleosts**. Funding Source:** NSERC Research Discovery Grant 2015-2020. **PI:** PM Craig. **Amount:** *$145,000*. **Relationship to Current Proposal:** This research program examines the impact of multiple stressors (aquatic contaminants, temperature, oxygen) on model (zebrafish) and non-model (rainbow darters) aquatic species. Initial experiments already conducted indicate that there is a substantial impact on the abundance of specific microRNA and the functional, phenotypic consequences of multi-stressor exposure in both zebrafish and darters. There is no budgetary overlap with the requested funds, although the methodological approaches and data generated through microRNA profiling, next generation sequencing, and whole animal metabolic assessment align with to the current proposal

**3) Title:** Integrative responses of fish to environmental stressors. **Funding Source:** JELF-CFI and ORF; 2015-2017. **PI:** Paul M Craig. **Amount:** *$300,000*. **Relationship to Current Proposal:** These funds were used to purchase the following phenotypic and cellular infrastructure: Loligo complete swim tunnel system (170ml capacity) for zebrafish swimming performance and oxygen consumption; 24-channel optical fluorescence oxygen system for high through-put respiration measurements in developing embryos; Qubit aquatic monitoring and manipulation system, which is custom designed for manipulating and recording multiple parameters (oxygen, temperature) for chronic periods in aquatic systems; an Oroboros high resolution respirometry with fluorescent capacity system for precise recording of mitochondria respiration. These funds have already been spent, and thus do not overlap with the requested funds.

**4) Title:** Laboratory Start-Up Funds. **Funding Source:** University of Waterloo Biology Department; 2014-2019. **PI:** PM Craig. **Amount:** *$200,000*. **Relationship to Current Proposal:** These funds were used to purchase essential molecular, cellular, and aquatic infrastructure to outfit my laboratory. Equipment purchased includes western blotting apparatus, gradient thermocyclers, gel electrophoresis equipment, 96-well plate spectrophotometer (including spectra-drop RNA/DNA quantification), room temperature and refrigerated microcentrifuge, pH meters, incubation refridgerator, aquatic recirculation system capable of housing 5000 zebrafish, and recently a dissection scope with camera/video capacity. Funds from my Start-Up grant do not overlap with the requested funds in the current proposal.

## Key Publications

**Peer-reviewed publications: 31; Citations: >1040; h-index: 16; i10-index: 23 (Google Scholar®)**

**1)** Best C, Ikert H, Kostyniuk D, Craig PM, Navarro-Martin L, Marandel L, Mennigen JA. (2018) Epigenetics in teleost fish: From molecular mechanisms to physiological phenotypes. Comp. Biochem. Physiol. B. 224:210-244. **2**) Mehdi H, Dickson F, Bragg LM, Servos MR, Craig PM. (2017) Impacts of wastewater treatment plant effluent on energetics and stress response of rainbow darter in the Grand River watershed. Comp. Biol. Physiol. B 224:270-279 S. **3)** Kuc C, Richard DJ, Johnson S, Bragg LM, Servos MR, Craig PM. (2017) Rainbow trout exposed to benzo[a]pyrene yields conserved microRNA binding sites in DNA methyltransferases across 500 million years of evolution. Sci. Rep. 7:16843. **4)** Gilmour KM, Craig PM, Dhillon RS, Lau, GY, Richards JG. (2017). Regulation of energy metabolism during social interactions in rainbow trout: A role for AMP-activated protein kinase. Am. J. Physiol. Regul. Integr. Comp. Physiol. 313:R549-R559. **5)** Fitzpatrick J, Earn D, Bucking C, Craig PM, Nadella S, Wood C, Balshine S. (2016) Postcopulatory consequences of female mate choice in a fish with alternative reproductive tactics. Behav. Ecol. 27(1):312-320. **6)** Cameron BE, Craig PM, Trudeau VL. (2016) Implication of microRNA deregulation in the response of vertebrates to endocrine disrupting chemicals. Environmental Toxicology & Chemistry. Environ. Toxicol. Chem. 35(4):788-793. **7)** Craig PM, Trudeau VL, Moon TW. (2014) Profiling Hepatic microRNAs in Zebrafish: Fluoxetine Exposure Mimics a Fasting Response That Targets AMP-Activated Protein Kinase (AMPK). PloS One. 9(4): 1-11. **8)** Massarsky A , Strek L , Craig PM , Eisa-Beygi S , Trudeau VL , Moon TW. (2014) Acute embryonic exposure to nanosilver or silver ion does not disrupt the stress response in zebrafish larvae and adults. Sci Total Environ. 478: 133-140. **9)** Craig PM, Fitzpatrick J, Walsh P, Wood C, McClelland G. (2014) Coping with aquatic hypoxia: how the plainfin midshipman tolerates the intertidal zone. Env. Biol. Fish. 97:163-172. **10)** Lemoine CM, Bucking C, Craig PM, Walsh PJ. (2014) Divergent hypoxia tolerance in adult males and females of the plainfin midshipman. Physiol. Biol. Zool. 87(2): 325-333. **11)** Craig PM, Massarsky A, Moon TW. (2013) Understanding glucose uptake during methionine deprivation in incubated rainbow trout hepatocytes using a non-radioactive method. Comp. Biol. Physiol. B. 166(1):23-29. **12)** Bucking C , LeMoine CM **,** Craig PM, Walsh PJ. (2013) Nitrogen metabolism of the intestine during digestion in a teleost fish, the plainfin midshipman. J. Exp. Biol. 216: 2821-2832. **13)** Melvin SD, Lanctôt CM, Craig PM**,** Moon TW, Peru KM, Headly JV, Trudeau VL. (2013) Effects of naphthenic acid exposure on development and liver metabolic processes in anuran tadpoles. Environ. Poll. 117:22-27. **14)** Craig PM, Moon TW. (2013) Methionine restriction affects the phenotypic and transcriptional response of rainbow trout to carbohydrate-enriched diets. Brit. J. Nutr. 109:402-412. **15)** Craig PM**,** Moon TW. (2011) Fasted zebrafish mimic genetic and physiological responses in mammal: A model for obesity and diabetes? Zebrafish. 8(3):109-117. **16)** Polakof S , Panserat S , Craig PM , Martyres DJ , Plagnes-Juan E , Savari S, Aris-Brosou S, Moon TW. (2011) The metabolic consequences of hepatic AMP-kinase phosphorylation in rainbow trout. PloS one. 6(5):e20228. **17)** Craig PM, Wood CM , McClelland GB. (2010) Water chemistry alters gene expression and physiological end points of chronic waterborne copper exposure in zebrafish, *Danio rerio*. Environ. Sci. Technol. 44:2156-2162. **18)** Craig PM**,** Hogstrand C, Wood CM, McClelland GB. (2009) Gene expression endpoints following chronic waterborne copper exposure in a genomic model organism, *Danio rerio*. Physiol. Genomics 40(1):23-33. **19)** Fitzpatrick JL, Craig PM, Bucking C, Balshine S, Wood CM, McClelland GB. (2009) Sperm performance under hypoxic conditions in the intertidal fish *Porichthys notatus*. Can. J. Zool. 87:464-469. **20)** Craig PM, Galus M, Wood CM, McClelland GB. (2009) Dietary iron alters waterborne copper-induced gene expression in softwater acclimated zebrafish. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296(2):R362-73.

## Curriculum Vitae

**Name/Affiliation:** Timothy D. Jardine, Ph.D.

**Current Job Title:** Associate Professor, School of Environment and Sustainability, Toxicology Centre, U of S, 2012-present

### Key Awards and Distinctions

New Researcher Award – University of Saskatchewan, 2018. Presented annually to an outstanding new scholar in recognition of their significant contributions to knowledge or artistic creativity. One award is given each year to a new faculty member who is within 10 years of PhD completion.

Water Security Research Excellence Award – Global Institute for Water Security, 2016. One award is given annually amongst 100 researchers in the institute (www.usask.ca/water) – $1000 cash credit for professional development and a public lecture on World Water Day;

Best paper award nomination – Australian Rivers Institute, 2012 (for Jardine et al. 2012, Journal of Animal Ecology 81:310-322). Three nominations are given annually amongst 47 researchers in the institute (www.rivers.edu.au);

Seventeen invited seminars since 2014, including a plenary in Porto Seguro, Brazil, and departmental seminars at University of North Texas (Texas), Cary Institute for Ecosystem Studies (New York), University of Canterbury (New Zealand) and Griffith University (Australia).

Fellow – Canadian Rivers Institute (http://canadianriversinstitute.com/); Grant reviews for NSERC (Discovery and CRD programs), US National Science Foundation, NERC Urgency Program, and Swiss National Science Foundation; 63 manuscript reviews since 2014 for journals including Ecology Letters, Ecology, and Environmental Science and Technology; External examiner for students at University of Lethbridge and Rhodes University; Member of the selection committee for the Richard Playle Award for Outstanding Thesis in Ecotoxicology; External review of the environmental monitoring program developed by Sherritt International in response to the Obed Mountain Mine coal tailings spill in Apetowun Creek, AB (2013/2014). Session chair, organizing committee member and presentation judge for Canadian Ecotoxicity Workshop and Society for Freshwater Science annual meetings. Featured scientist in various print media outlets (2015-2019), including the Globe and Mail (Husky Oil Spill), Wildlife Society and WaterToday (Saskatchewan River Delta); Television interviews, including Global News Saskatoon (Saskatchewan River Delta), CTV Saskatoon (World Water Day, Husky Oil Spill), CTV Regina (Husky Oil Spill); Radio interview with CBC Regina (Husky Oil Spill); Six co-presentations with community partners since 2014, including one at the International Symposium on Society and Resource Management (Houghton, MI, June 2016).

### Research Funding

Career: $2.42 million in competitive grants as PI or Co-PI, including funds from NSERC (Discovery, Collaborative Research and Development, and Research Tools and Instruments), SSHRC (Partnership Development and Connection) and CIHR (Network Environments for Indigenous Health Research).

## Most Significant Contributions

1. ***Hydrological connectivity and ecosystem function.*** My work with ecological tracers in free-flowing rivers helps us understand the importance of water-mediated transfers of nutrients and energy in shaping food web structure and function [Jardine et al. 2015a Ecology 96:684-692]. In hydrologically dynamic systems, temporarily inundated floodplains supply a disproportionate amount of food for large predatory fishes that are economically and culturally important to Indigenous communities [Jardine et al. 2012 Oecologia 168:829-838]. The use of these non-local resources also has implications for nutrient cycling and cross-ecosystem subsidies [Jardine et al. 2015b Ecology 96:3257-3269]. Animals that can move throughout river networks are more weakly coupled to the local prey base because they do their foraging elsewhere, acting as vectors of nutrient movements [Jardine et al. 2012 J. Anim. Ecol. 81:310-322].

2. ***Monitoring long-term social-ecological change in large rivers.*** The logical extension of the above expertise is my ongoing work in large river deltas that have experienced changes in the quality, quantity and seasonal timing of water reaching these vital floodplain ecosystems. Much of this activity involves the development of robust and credible indicators of ecosystem health. In response to concerns from local communities (Kowanyama in northern Australia, Cumberland House in SK, Fort Smith and Fort Resolution in NWT) about upstream contaminant sources and river regulation, we used remote-sensing of flood areas [Ward et al. 2013 Ecohydrology 6:312-323] to show how dam-induced declines in connectivity [Sagin et al. 2015 Int. J. Remote Sens. 36:764-781] affects water quality and fish habitat [MacKinnon et al. 2016 Can. J. Fish. Aquat. Sci. 73:140-152] and how that might change further in the future [Hassanzadeh et al. 2017 Ecohydrology 10 :e824]. We applied innovative Bayesian Belief Models to unify scientific data and Traditional Knowledge in a single framework [Mantyka-Pringle et al. 2017, Environ. Int. 102 : 125-137].

3. ***Contaminant sources and fate in freshwaters.*** Mercury and some organic chemicals biomagnify to unsafe concentrations in aquatic food webs. My work evaluates global drivers of food web biomagnification of mercury and persistent organic pollutants [Walters et al. 2016 Env. Sci Tech. 50:4650-4658] using comparative approaches in warm and cool regions of the world. Our global analysis of this phenomenon for mercury [Lavoie et al. 2013 Env. Sci. Tech. 47:13385-13394] showed how Arctic ecosystems are more vulnerable to biomagnification, and was highlighted in the news section of Science Magazine (Fahrenkamp-Uppenbrink, 15-Nov-2013, Vol. 342, pg. 779); this paper has already been cited 228 times (Web of Science). Following from this work, my laboratory showed that beaver ponds act as sources of mercury to downstream ecosystems [Painter et al. 2015 Ecosphere 6:194], a phenomenon we also demonstrated with hydroelectric dams [Green et al. 2016 Arch. Env. Contam. Toxicol. 71:157-170].

4. ***Identifying and overcoming limitations of stable isotope techniques in ecology.*** The application of stable isotope techniques to ecological studies is a broad and growing research area; many new users need proper guidance. I have been a leader in documenting and addressing caveats associated with the technique for several years [Jardine et al. 2006 Env. Sci. Tech. 40:7501-7511], most recently offering guidance in study design [Jardine et al. 2014 River Res. Appl. 30:155-165] and evaluating isotopic approaches to examine individual dietary specialisation [Bond et al. 2016 Methods Ecol. Evol. 7:1428-1437]. In recognition of my expertise in this area, I was recently invited to write a book chapter describing the use of isotopes in food webs [Jardine et al. in press, in Food Forensics – Stable Isotopes as a Guide to Authenticity and Origin].

## Relationship to Other Grants

**1) Title:** Can isolated wetlands come to the trophic rescue of surrounding ecosystems? Investigating new pathways for food web connectivity. **Funding Source:** NSERC Discovery, 2018 - 2023. **PI:** Jardine, T.D. **Amount:** $165,000. **Relationship to Current Proposal:** This project is examining biological links between wetlands and rivers. There is no conceptual or fiscal overlap with the current proposal.

**2) Title:** Enhancing institutional connectivity to study biological connectivity in the world's great rivers. **Funding Source**: U of S International Research Partnership Fund. 2018-2019. **PI:** Jardine, T.D. **Amount**: $20,000. **Relationship to Current Proposal:** This is for food web research in the Amazon basin in Brazil. There is no conceptual or fiscal overlap with the current proposal.

**3) Title:** Indigenous Water and Health Reconciliation Network (IWHR2N). **Funding Source:** CIHR Network Environments for Indigenous Health Research. 2018-2019. **PI:** Bharadwaj, L. (T.D. Jardine CoI). **Amount:** $75,000. **Relationship to Current Proposal:** This project connects academics and communities to better advance water and health research. There is no conceptual or fiscal overlap with the current proposal.

**4) Title:** Evaluating effects of the Husky Energy oil spill on fishes in the North Saskatchewan River**.** **Funding Source:** Department of Fisheries and Oceans, Canada, 2017-2020. **PI:** T. Jardine (J.P. Giesy CoI). **Amount:** $224,250. **Relationship to Current Proposal**: This project is investigating concentrations of PAHs in fishes and assessing potential effects. Some collaborative work was conducted in Phase 1 to compare eDNA assessments of fish communities with traditional survey techniques, but there is no additional overlap planned for phase 2.

**5) Title:** Integrated modelling for prediction and management of change in Canada's major river basins. **Funding source:** Global Water Futures. **PI:** S. Razavi (T. Jardine CoI). **Amount:** $1,650,000. **Relationship to Current Proposal:** My role in this consortium is to assist in the development of environmental flows. While there may be some geographic overlap in the river basins chosen in each program, there is no fiscal relationship between proposals.

**6) Title:** We need more than just water: Assessing sediment limitation in a large freshwater delta. **Funding Source:** Global Water Futures, 2019 - 2021. **PI:** Jardine, T.D. (G. Carriere co-PI). **Amount:** $199,980. **Relationship to Current Proposal:** This project is examining sediment transport and chemical characteristics of deposited sediment in the Saskatchewan River basin. There is no conceptual or fiscal overlap with the current proposal.

**1) Title:** Indigenous engagement on Lake Winnipeg basin nutrient issues in Treaty 4, 5, and 6, Saskatchewan. **Funding Source:** Lake Winnipeg Basin Program, Environment Canada, 2019 - 2022. **PI: L. Bradford (**Jardine, T.D. **Amount:** $177,805. **Relationship to Current Proposal:** This project is examining nutrients flows to and from Indigenous communities in the Saskatchewan River basin. There is no conceptual or fiscal overlap with the current proposal.

## Key Publications

**Peer-reviewed publications: 78; Citations: 3200; h-index: 28; i10-index: 50 (Google Scholar®)**

1. Jardine, T.D., Doig, L.E., Jones, P.D., Bharadwaj, L., Carr, M., Tendler, B., and Lindenschmidt, K.-E. 2019. Vanadium and thallium exhibit biodilution in a northern river food web. Chemosphere 233: 381-386.
2. Yates, A.G., Culp, J.M., Armanini, D.G., Baird, D.J., Jardine, T.D., and Orlofske, J. 2019. Enhancing bioassessment approaches: Development of a river services assessment framework. Freshwater Science 38: 12-22.
3. Hassanzadeh, E., Elshorbagy, A., Nazemi, A., Jardine, T.D., Wheater, H., and Lindenschmidt, K.-E. 2017. The eco-hydrological vulnerability of a large inland delta to changing regional streamflows and upstream irrigation expansion. Ecohydrology 10: e1824.
4. Mantyka-Pringle, C.S., Jardine, T.D., Bradford, L.E., Bharadwaj, L., Kythreotis, A.P., Fresque-Baxter, J., Kelly, E., Somers, G., Lindenschmidt, K.-E., Doig, L.E., Jones, P.D., and the Slave River and Delta Partnership. 2017. Bridging science and traditional knowledge to assess cumulative impacts of stressors on aquatic ecosystems. Environment International 102: 125-137.
5. Walters, D.M., Jardine, T.D., Cade, B., Kidd, K.A., Muir, D.C.G., Leipzig-Scott, P. 2016. Trophic magnification of organic chemicals - a global synthesis. Environmental Science and Technology 50: 4650-4658.
6. Jardine, T.D., Woods, R., Marshall, J., Fawcett, J., Lobegeiger, J., Valdez, D., and Kainz, M.J. 2015. Reconciling the role of organic matter pathways in aquatic food webs by measuring multiple tracers in individuals. Ecology 96: 3257-3269.
7. Jardine, T.D., Bond, N.R., Burford, M.A., Kennard, M.J., Ward, D.P., Bayliss, P., Davies, P.M., Douglas, M.M., Hamilton, S.K., Melack, J.M., Naiman, R.J., Pettit, N.E., Pusey, B.J., Warfe, D.M., and Bunn, S.E. 2015. Does flood rhythm drive ecosystem responses in tropical riverscapes? Ecology 96: 684-692.
8. Painter, K.J., Westbrook, C.J., Hall, B.D., O'Driscoll, N.J., and Jardine, T.D. 2015. Effects of in-channel beaver impoundments on mercury bioaccumulation in Rocky Mountain stream food webs. Ecosphere 6: 194.
9. Jardine, T.D. 2014. Organic matter sources and size structuring in stream invertebrate food webs across a tropical to temperate gradient. Freshwater Biology 59: 1509-1521.
10. Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., and Campbell, L.A. 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. Environmental Science and Technology 47: 13385-13394.
11. Jardine, T.D., Kidd, K.A., and Rasmussen, J.B. 2012. Terrestrial organic matter in the diet of stream consumers: Implications for mercury exposure. Ecological Applications 22: 843-855.
12. Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., and Lutcavage, M.E. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modeling methods. Journal of Animal Ecology 77: 838-846.
13. Jardine, T.D., Kidd, K.A., and Fisk, A.T. 2006. Applications, considerations and sources of uncertainty when using stable isotope analysis in ecotoxicology. Environmental Science and Technology 40: 7501-7511.

## Curriculum Vitae

**Name/Affiliation:** Andrew C. DOXEY, Ph.D., University of Waterloo

### Current Job Title

2019-Present Associate Professor (full-time, tenure-track), Dept. of Biology (cross-appointed to School of Computer Science), University of Waterloo
2018-Present Assistant Clinical Professor (Adjunct), Dept. of Medicine, McMaster University

### Key Awards and Distinctions

- 2018 Thermo Fisher award for Outstanding Contributions to Microbiology
- 2018 Science Excellence in Early Career Research Award (SEECRA), University of Waterloo
- 2018 Outstanding Performance Award, University of Waterloo
- 2017 Ontario Early Researcher Award (ERA)
- 2010-2012 NSERC Postdoctoral Fellowship (PDF)
- 2010 Governor General's Gold Medal, top Ph.D. at University of Waterloo
- Published 43 peer reviewed journal articles, which have received 870 citations, with a Hirsch (H) index of 17 (Google Scholar®)
- Dissemination of research through presentations at national and international conferences (Total: 53 conference proceedings, 26 invited talks)
- Recognized as a leader in my field; active in the International Society for Computational Biology (ISCB), Canadian Society for Microbiology (CSM), and International Neurotoxin Association (INA); reviewer for national granting agencies and various journals in Bioinformatics, Genetics and Molecular Biology

### Research Funding

Current funding:

- Targeted discovery of novel microbial proteolytic enzymes in biofilm and host tissue degradation, Ontario Early Research Award (ERA), 2017-2022, $150,000 (sole PI)
- Detecting functional novelty in microbial genomes: discovery and characterization of new metalloprotease and toxin families, NSERC Discovery Grant and DAS award, 2013-2018, $250,000 + $120,000 (sole PI)
- Analysis of Nucleic Acids from Difficult Media: Method Optimization for Bentonite Clays and Rock Cores Associated with Deep Geological Repositories for Nuclear Waste, NSERC Collaborative Research and Development (CRD) Grant, 2018-2021, $1,000,000 (co-Lead w/ J. Neufeld)
- Defining the impacts of cannabis smoke exposure on respiratory mucosal immunity, Ontario Lung Association Cannabis and Lung Health Research Grant, 2019-2020, $50,000 (Co-Applicant w/ J. Hirota)

Completed funding:

- Detecting protein structural and functional innovations from genomic data. NSERC Discovery Grant. 2013-2019. $205,000 (sole PI)

**Most Significant Contributions** (references[x] correspond to Key Publications)

Research in the Doxey lab is at the forefront of applied bioinformatics, combining both computational and experimental approaches to discover novel protein functions and evolutionary adaptations from genomes, with a focus on pathogens, host-associated microbes, and unexplored molecular diversity.

**Computational prediction of novel protein families and functions.** Dr. Doxey has made several important contributions in the area of protein function prediction, including new tools and algorithms for identifying biosynthetic enzymes[16], virulence factors and protein toxins[3-7,18]. The central theme of these tools is the use of pattern-detection techniques and integration of various 'omic data sources including genomic, proteomic, gene expression, and large-scale protein structural information. Recent genomics- and bioinformatics-driven discoveries by Doxey Lab tools include the discovery of novel botulinum neurotoxins[14], metalloproteases[13], cobalamin biosynthetic enzymes[16], and the first family of enzymatic flagellins[8].

**Detection of evolutionary adaptations in genes and genomes.** Dr. Doxey's group has also developed new methods to detect adaptive events in genes and genomes. Estimating the functional effects of sequence mutation, whether subtle or dramatic, is key in efforts to functionally interpret new genome sequences. By combining sequence analysis, phylogenetics and structural modeling, new methods developed by the Doxey lab are capable of identifying the critical mutations in gene families that alter molecular function (e.g., binding specificity, protein-protein interactions and enzymatic activity)[9]. These approaches have been used to reconstruct the evolution of function in thousands of gene families spanning bacteria, plants, protozoans, and vertebrates. This work has also generated important insights into the *de novo* evolution of protein folds and the evolutionary origins of bacterial virulence factors[3].

**Comparative functional genomics/metagenomics.** The Doxey Lab has made important contributions in genomics, having developed several new computational methods for functional annotation, visualization and comparison of microbial genomes and metagenomic datasets. Recent work includes the development of AnnoTree, the first functionally annotated, interactive microbial tree of life[1], and the MetAnnotate framework[12] (metannotate.uwaterloo.ca) for combined taxonomic and functional profiling of metagenomic datasets. This pipeline is among the first automated pipelines for analyzing metagenomic datasets to determine not only "who is there" (taxonomic composition of microbial community) but, importantly, "what are they doing". In addition, the Doxey lab has performed the first large-scale characterization of metagenomic "ORFans" – gene families that can be identified in metagenomes but lack any detectable similarity to known genes[13]. The identified genes encode thousands of novel proteins and enzymes requiring experimental characterization.

The Doxey lab's metagenome annotation pipelines have also led to the groundbreaking discovery of global vitamin $B_{12}$ production by marine *Thaumarchaeota*[16]. This discovery has major implications for global biogeochemistry and marine microbial ecology, and serves as the basis for several ongoing projects on metagenomics analysis of marine nutrient cycling.

**Molecular mimicry of host proteins by pathogenic bacteria.** It is well established that virulence factors encoded by bacterial pathogens often structurally mimic key host proteins. Dr. Doxey developed the first approach to detect potential mimicry relationships in bacterial pathogens, and has screened hundreds of bacterial genomes for novel virulence factors. This work has detected known and novel mimicry related virulence mechanisms of bacterial pathogens of humans[18], and become implemented as a web-based tool[15] (mimicme.uwaterloo.ca) to serve the broader research community. New virulence factors predicted by the method have since been validated by experimental studies and shown to play major roles in bacterial pathogenesis (Grover et al., 2014; *Science*, 343:656-661).

## Relationship to Other Grants

There is no budgetary overlap between any current or future funding sources. As described below, the following grants align with the goals of the current CFREF proposal.

**1) Title:** Detecting functional novelty in microbial genomes: discovery and characterization of new metalloprotease and toxin families. **Funding Source:** NSERC (Discovery Grant); April 1, 2019 – March 31, 2024. **PI:** AC Doxey. **Amount:** $250,000 + 3-year Discovery Accelerator Award ($120,000). **Relationship to Current Proposal:** These funds provide support for salaries, equipment and reagent costs supporting three ongoing research themes in the Doxey lab: 1) Development of computational tools for microbial genome data mining; 2) Prediction of novel protease and toxin families; 3) Biochemical testing of predicted protein functions and functional shifts, with an emphasis on proteolytic enzymes. There is no budgetary overlap with the requested CFREF funds, although the methodological approaches developed for analysis of metagenomic/next-generation sequencing align strongly with the goals of the current proposal.

**2) Title:** Targeted discovery of novel microbial proteolytic enzymes in biofilm and host tissue degradation. **Funding Source:** Ontario Ministry of Research, Innovation, and Science; April 1, 2017 – March 31, 2022. **PI:** AC Doxey. **Amount:** $150,000. **Relationship to Current Proposal:** These funds provide support for salaries supporting a technician and two Ph.D. students to conducting research on the metagenomic characterization of microbial communities during biofilms and animal tissue degradation. Research aims of the project are to characterize microbial community succession over space and time in developing and mature mixed-species biofilms as well as decomposing vertebrate tissues using high-throughput amplicon and metagenomic sequencing. Identified proteolytic enzymes of interest with novel roles in tissue degradation will be expressed, purified, and tested using biochemical approaches. There is no budgetary overlap with the requested CFREF funds. However, the further development metagenomic/next-generation sequencing analysis pipelines made possible by this program will support the proposed work.

**3) Title:** Analysis of Nucleic Acids from Difficult Media: Method Optimization for Bentonite Clays and Rock Cores Associated with Deep Geological Repositories for Nuclear Waste. **Funding Source**: NSERC Collaborative Research and Development (CRD) Grant; 2018-2021. **PI**: J Neufeld, AC Doxey. **Amount**: $1,000,000. **Relationship to Current Proposal:** This work involves metagenomic characterization of deep subsurface environments and assessment of their potential impact on nuclear waste repositories. There is no budgetary overlap with the requested CFREF funds. However, the further development metagenomic/next-generation sequencing analysis pipelines made possible by this program will support the proposed work.

## Key Publications

**Peer-reviewed publications: 43; Citations: 870; h-index: 17; i10-index: 25 (Google Scholar®)**

1. Mendler K, Chen H, Parks DH, Hug LA, **Doxey AC**. 2019. AnnoTree: visualization and exploration of a functionally annotated microbial tree of life. *Nucleic Acids Research*, 47:4442-4448.
2. Bergstrand LH, Neufeld JD, **Doxey AC**. 2019. Pygenprop: a Python library for programmatic exploration and comparison of organism Genome Properties. *Bioinformatics*, pii: btz522.
3. **Doxey AC**, Mansfield MJ, Lobb BA. 2019. Exploring the Evolution of Virulence Factors through Bioinformatic Data Mining. *mSystems*, 4:e00162-19.
4. Mansfield MJ, Wentz TG, Zhang S, Lee EJ, Dong M, Sharma SK, **Doxey AC**. 2019. Bioinformatic discovery of a toxin family in *Chryseobacterium piperi* with sequence similarity to botulinum neurotoxins. *Scientific Reports*, 9:1634.
5. Mansfield MJ, Sugiman-Marangos S, Melnyk RA, **Doxey AC**. 2018. Identification of a diphtheria toxin like gene family beyond the Corynebacterium genus. *FEBS Letters*, 592:2693-2705.
6. **Doxey AC**, Mansfield MJ, Montecucco C. 2018. Discovery of novel bacterial toxins by genomics and computational biology. Toxicon, 147:2-12
7. Zhang S, Lebreton F, Mansfield MJ, Miyashita S, Zhang J, Schwartzman JA, Tao L, Masuyer G, Martinez-Carranza M, Stenmark P, Gilmore MS, **Doxey AC***, Dong M*. Identification of a Botulinum Neurotoxin-like Toxin in a Commensal Strain of *Enterococcus faecium*. Cell Host & Microbe, 23:1-8.
8. Eckhard U, Bandukwala H, Mansfield MJ, Marino G, Cheng J, Wallace I, Holyoak T, Charles TC, Austin JW, Overall CM, **Doxey AC**. 2017. Discovery of a proteolytic flagellin family in diverse bacterial phyla that assembles enzymatically active flagella. *Nature communications*, 8:521.
9. Adams J, Mansfield MJ, Richard DJ, **Doxey AC**. 2017. Lineage-specific mutational clustering in protein structures predicts evolutionary shifts in function. *Bioinformatics*, 33:1338-1345.
10. Capellini TD, Chen H, Cao J, **Doxey AC**, Kiapour K, Schoor M, Kingsley DM. 2017. Ancient selection for derived alleles at a GDF5 enhancer influencing human growth and osteoarthritis risk. *Nature Genetics*, 49:1202–1210.
11. Lobb B, **Doxey AC**. 2016. Novel function discovery through sequence and structural data mining. *Current Opinion in Structural Biology*, 38:53-61.
12. Petrenko P, Kurtz DA, Lobb B, Neufeld JD, **Doxey AC.** 2015. MetAnnotate: function-specific taxonomic profiling and comparison of metagenomes. *BMC Biology*, 13:92.
13. Lobb B, Kurtz D, Moreno-Hagelsieb G, **Doxey AC**. 2015. Remote homology and the functions of metagenomic dark matter. *Frontiers in Microbiology*, 6:234.
14. Mansfield M, Adams J, **Doxey AC**. 2015. Botulinum neurotoxin homologs in non-*Clostridium* species. *FEBS Letters*, 589:342-8
15. Petrenko P, **Doxey AC**. 2015. mimicMe: a webserver for prediction and analysis of host-like proteins in microbial pathogens. *Bioinformatics*, 31:590-592.
16. **Doxey AC**, Kurtz D, Sauder L, Lynch MDJ, Neufeld JD. 2015. Aquatic metagenomes implicate *Thaumarchaeaota* in global cobalamin production. *ISME J*, 9:461-71.
17. Guturu H*, **Doxey AC***, Wenger AM, Bejerano G. (2013) Structure-aided prediction of transcription factor complexes in conserved non-coding elements. *Philosophical Transactions of the Royal Society B*, 368. * Co-first authors.
18. **Doxey AC**, McConkey BJ. 2013. Prediction of molecular mimicry candidates in human pathogenic bacteria. *Virulence*, 4:453-66.

# Curriculum Vitae

**Name/Affiliation:** John P. Giesy, Ph.D., FRSC, FSETAC,

**Current Job Title:** Professor & Canada Research Chair in Environmental Toxicology, 2006-Present

## Key Awards and Distinctions

- 1993 International Man of the Year-Environmental Toxicology; Title of Distinguished Professor from Michigan State University; Sigma Xi Meritorious Research Award
- 1994 Willard F. Shepard Award from the Michigan Water Pollution Control Association; Vollenweider Medal for Aquatic Sciences from the National Water Research Institute of Canada for his work on contaminants in the North American Great Lakes; QUINTESSENCE Award: Excellence in Environmental Contamination & Toxicology for a published paper; Chevron Distinguished Lectureship Award for research on the toxic effects of environmental contaminants on wildlife; CIBA-GEIGY Agricultural Recognition Award for work on microcosms and pesticides
- 1995 Founders Award, the highest award of the Society of Environmental Toxicology and Chemistry (SETAC)
- 1996 Distinguished Alumni Award from Alma College
- 2002 SETAC/Menzie-Curra Environmental Education Award from for his many activities in environmental education, including undergraduate and graduate training
- 2003 Sir E.W. Russell Award in the Sciences from the British Soil Science Society
- 2009 Named Einstein Professor, Chinese Academy of Sciences
- 2010 Distinguished Visiting Professor Award, King Saud University
- 2010 Elected fellow of Royal Society of Canada in Division III (National Academy of Science)
- 2013 University of Saskatchewan J.W. George Ivany Internationalization Award; Miroslaw Romanowski Medal from Royal Society of Canada; Society of Environmental Toxicology and Chemistry Capacity-Building Award
- 2015 Water Security Research Excellence Award from Global Institute for Water Security, University of Saskatchewan; Science Ambassador to China, University of Saskatchewan; Elected Fellow of Society of Environmental Toxicology and Chemistry
- 2016 University of Saskatchewan Distinguished Researcher Award
- 2018 Erasmus Award, European Union https://czs.muni.cz/en/staff-from-abroad/teaching-at-mu/erasmus-icm
- 2019 Honorary Degree Doctor *Honoris Causa* in the field of Environmental Sciences, conferred by Masaryk University, Czech Republic.

## Research Funding

Career: $245,049,190 CAD from local, state, provincial, federal, foundations and international agencies and organizations. Current funding is listed in the section on Relationship to other funding.

## Relationship to Other Grants

**1) Title:** Toxico-genomic Assessment of Emerging Environmental Pollutants Using Novel Functional Genomic and High Throughput Technologies. **Funding Source:** European Commission (The) program on Solutions for present and future emerging pollutants in land and water resources management, 2013-2018. **PI:** Xiaowei Zhang (Giesy CoI). **Amount:** $453,270. **Relationship to Current Proposal:** This project is funded to Nanjing University where I am a concurrent professor and help supervise post docs and students. We are developing ecogenomic approaches to assessing the environment. This project is allowing us to develop technologies that can be applied in the current project, but no funds can be transferred to Canada so there is no fiscal overlap with the current proposal.

**2) Title:** Novel Natural and Synthetic Brominated and Iodinated Compounds in the Environment**. Funding Source**: Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant, 2017-2022 (Pending). **PI:** J.P. Giesy. **Amount**: $200,000. **Relationship to Current Proposal:** None

**3) Title:** Potential Impacts of Modern Perfluorinated Chemicals on Fisheries. **Funding Source:** Department of Fisheries and Oceans, Canada", 2017-2020 (Pending). **PI:** P.D. Jones (J.P. Giesy CoI). **Amount:** $257,600. **Relationship to Current Proposal:** This project is to assess toxic potencies of Perfluorinated compounds on arctic fishes and has no overlap with the proposal to GWF.

**4) Title:** Evaluating effects of the Husky Energy oil spill on fishes in the North Saskatchewan River**. Funding Source:** Department of Fisheries and Oceans, Canada, 2017-2020. **PI:** T. Jardine (J.P. Giesy CoI). **Amount:** $224,250. **Relationship to Current Proposal**: This project is to investigate concentrations of residues in fishes and assess potential effects. **Relationship to Current Project**: There is no overlap with the proposal submitted to the GWF.

**5) Title:** "Omic' and chemical fingerprinting methodologies using ultrahigh-resolution mass spectrometry for geochemistry and healthy waters". Funding Source: National Science and Engineering Research Council of (NSERC), Canada First Research Excellence Fund (CFREF. "Global Waters Future Pillar 1". May 2018-April 2021. ($250,000 CAD) **PI:** P.D. Jones, J.P. Giesy (Co-I), Giesy portion $125,000. **Relationship to Current Proposal**: This project is to develop chemical omics techniques and investigate concentrations of residues in surface waters and fishes and assess potential effects. The results of this study can be leveraged against the results of the proposed e-DNA studies.

**6) Title:** "Assessment of Occurrence of Synthetic Hormone [melengestrol Acetate (MGA), Trenbolone Acetate (TBA)] and Beta-agonist (ractopamine) in Cattle Operations and Associated Environments". 2018-2021. **Funding Agency:** Beef Cattle Research council of Canada. Total Funding: ($502,000 CAD), $376,500. Funding in 2018-2019: $94,125. **PI:** J. Giesy, P.D. Jones (Co-I). **Relationship to Current Project:** None

## Most Significant Contributions

I am an environmental toxicologist with interests in many aspects of this field, including both fates and effects of potentially toxic compounds and elements, particularly in the area of ecological risk assessment, aquatic toxicology, wildlife and avian toxicology. I have conducted research into the movement, bioaccumulation, and effects of toxic substances at different levels of biological organization, ranging from biochemical to ecosystem. I have done extensive research in the areas of metal speciation, multispecies toxicity testing, biochemical indicators of stress in aquatic organisms, fate, and effects of PAHs, halogenated hydrocarbons, including chlorinated dibenzo-dioxins and -furans, PCBs and pesticides. In addition to my work in aquatic toxicology, I have become world-famous for my studies on wildlife toxicology, particularly in the area of endocrine modulating compounds. In addition to my work

as an ecologist and biochemical toxicologist I am a world-class environmental chemist, having developed and applied both analytical and bio-analytical techniques to environmental issues.

1. I determined the cause of deformities and embryo-lethality of birds in the North American Great Lakes.
2. I determined causes of decreased reproduction of salmonid fishes of the Great Lakes and Baltic Sea.
3. I discovered the phenomenon of photo-enhanced toxicity of organic compounds, such as PAHs.
4. I was the first to discover perfluorinated compounds in the environment, an important new class of environmental contaminants that are now listed on the Stockholm Convention for Persistent Organic Pollutants and 87 individual compounds are now banned in Canada
5. I developed and patented the *H295R Steroidogenesis Assay*, which is now required by the US EPA to screen all chemicals used in commerce for potential endocrine disrupting effects. The assay is now approved by OECD for use internationally.

## Key Publications

**Peer-reviewed publications:1,235; Citations:>65,895; h-index:120; i10-index:855 (Google Scholar®)**

**1.** Yang, J.-H., X.-W. Zhang, Y.-W. Xie, C. Song, J.-Y. Sun, Y. Zhang, J.P. Giesy and H.-X. Yu. 2017. Ecogenomics of Zooplankton Community Reveals Ecological Threshold of Ammonia Nitrogen. *Environ. Sci. Technol.* DOI: 10.1021/acs.est.6b05606

**2.** Xie, Y.-W., S.-G Hong, S.-J. Kim, X.-W. Zhang, J.-G. Yang, J.P. Giesy, T.-Y. Wang, Y.-L. Lu, H.-X. Yu, J.-S. Khim. 2017. Ecogenomic Responses of Benthic Communities under Multiple Stressors along the Marine and Adjacent Riverine Areas of Northern Bohai Sea, China. *Chemosphere*. 172:166-174.

**3.** Xie Y.-W., J.-H Wang, J.-H Yang, J.P. Giesy, H.Yu and X.-W. Zhang. 2017. Environmental DNA Metabarcoding Reveals Primary Chemical Contaminants in Freshwater Sediments from Different Land-Use Types. Chemosphere. 172:201-209.

**4.** Xie, Y-W., P. Xia, H. Wang, H.-X. Yu, J.P. Giesy, Y. Zhang, M.A. Mora and X.-W. Zhang. 2016. Effects of Captivity and Artificial Breeding on Microbiota in Feces of the Red-crowned Crane (*Grus japonensis*). Scientific Reports 6:33350.

**5.** Wang, X.-X. X.-W. Zhang, P. Xia, J.-J. Zhang, Y.-T. Wang, R. Zhang, J.P. Giesy, W. Shi and H.-X. Yu. 2017. A High-through-put, Computational System to Predict if Environmental Contaminants Can Bind to Human Nuclear Receptors. *Sci. Total Environ.* 576:609–616.

**6.** Chen, L.-G., D.W.T. Au, C.-Y. Hu, W.-P. Zhang, B.-S. Zhou, L. Cai, J.P. Giesy and P.-Y. Qian. 2017. Linking Genomic Responses of Gonads with Reproductive Impairment in Marine Medaka (*Oryzias melastigma*) Exposed Chronically to the Chemopreventive and Antifouling Agent, 3,3'-diindolylmethane (DIM). *Aquatic Toxicol.* 183:135–143.

**7.** Jeon, S.-Y., S.-J. Hong, B.-O. Kwon, J.-S. Park, S.-J. Song, J.P. Giesy and J.-S. Khim. 2017. Assessment of Potential Biological Activities and Distributions of Endocrine-disrupting Chemicals in Sediments of the West Coast of South Korea. *Chemosphere.* 168:441-449.

**8.** Peng, H., D.M.V. Saunders, J.-S. Sun, P.D. Jones, C.K.C. Wong, H.-L. Liu and J.P. Giesy. 2017. Mutagenic Azo Dyes, Rather than Flame Retardants, are Predominant Brominated Compounds in House. *Envir. Sci. Technol*. 50:12669−12677.

**9.** Chen, L., Y.-F. Hu, J. He, J. Chen, J.P. Giesy and P. Xie. 2016. Responses of the Proteome and Metabolome in Livers of Zebrafish Exposed Chronically to Environmentally Relevant Concentrations of Microcystin-LR. *Envir. Sci. Technol.* . 51:596-607.

**10.** Zhang, Z.-H., S. Tang, M. Wang, W.-M. Sun, Y.-W. Xie, H. Peng, A. Zhong, H.-L. Liu, X.-W. Zhang, H.-X. Yu, J.P. Giesy and M. Hecker. 2019. Acid Mine Drainage Affects the Diversity and Metal Resistence Gene Profile of Sediment Bacterial Community Along a River. *Chemosphere*. 217:790-799.

**11.** Jung, D.-W., M. Guan, S.-W. Lee, C.-M. Kim, H.-S. Shin, S.-J. Hong, U.-H. Yim, W.-J. Shim, J. P. Giesy, J.-S. Khim, X.-W. Zhang and K.-H. Choi. 2016. Searching for Novel Modes of Toxic Actions of Oil Spill using *E. coli* Live Cell Array Reporter System – A Hebei Spirit Oil Spill Study. *Chemosphere.* 169:669-677.

**12.** Sun, J.-X., S. Tang, H. Peng, D.M.V. Saunders, J.A. Doering, M. Hecker, P.D. Jones, J. P. Giesy and S. Wiseman. 2016. Combined Transcriptomic and Proteomic Approach to Identify Toxicity Pathways in Early Life Stages of Japanese Medaka (*Oryzias latipes*) Exposed to 1,2,5,6-Tetrabromocyclooctane (TBCO). *Envir. Sci. Technol.* 50:7781-7790.

**13.** Shi W, D. Deng, Y. Wang, G. Hu, J. Guo, X. Zhang, X. Wang, J.P. Giesy, H. Yu and Z. Wang. 2015. Causes of Endocrine Disrupting Potencies in Surface Water in East China. *Chemosphere.* 144:1435-1442.

**14.** Chen, L.G., J.C.W. Lam, C.-Y. Hu, M.M.P. Tsui, Q. Wang, J.P. Giesy and P.K.S. Lam. 2018. Perfluorobutanesulfonate Exposure Causes Durable and Transgenerational Dysbiosis of Gut Microbiota in Marine Medaka. Envir. Sci. Tech. Let. 5:731-738.

**15.** Zhang, C., W.-Y. Feng, H.-Y. Cheng, Y.-R. Zhu, F.-C. Wu, J.P. Giesy, Z.-Q. He, H. Wang and F.-H. Sun. 2018. Characterization and Sources of Dissolved and Particulate Phosphorus in 10 Freshwater Lakes with Different Trophic Statuses in China by Solution 31P Nuclear Magnetic Resonance Spectroscopy Ecol. Res. 34:106–118.

**16.** Doering, J.A., S. Wiseman, J.P. Giesy and M. Hecker. 2018. Cross-Species Quantitative Adverse Outcome Pathway for Activation of the AHR Leading to Early Lifestage Mortality in Birds and Fishes. *Envir. Sci. Technol*. 52-7524-7533.

**17.** Chen, Q.-C. X.-X. Wang, H.-Y. Tan, W. Shi, X.-W. Zhang, S. Wei, J.P. Giesy and H.-X. Yu. 2019. . Molecular Initiating Events of Bisphenols on AR-Mediated Pathways Provide Guidelines for *in Silico* Screening and Design of Substitute Compounds. *Envir. Sci. Technol. Let.* 6:205−210.

**18.** Liu, H.-L., L.-H. Shi, J.P. Giesy and H.-X. Yu. 2019. Polychlorinated Diphenyl Sulfides Induce ROS and Genotoxicityvia the AhR-CYP1A1 Pathway and Cause Genotoxicity. *Chemosphere* 223:165-170.

**19.** Liu, H.-L., Z.-Y. Ma, T. Zhang, N.-Y. Yu, G.-Y. Su, J.P. Giesy and H.-X.Yu. 2018. Pharmacokinetics and Effects of Tetrabromobisphenol a (TBBPA) to Early Life Stages of Zebrafish (*Danio rerio*). *Chemosphere* 190:243-252.

**20.** Al Naggar, Y., J.P. Giesy and S. El Kholy. 2019. Sublethal Effects of Chronic Exposure to Chlorpyrifos or Imidacloprid Insecticides or their Binary Mixtures on *Culex pipiens Mosquitoes. Physiol. Entomol.* 44:123-132. ing and Design of Substitute Compounds. *Envir. Sci. Technol. Let.* 6:205−210.

**21.** Peng, Y., J.-P. Wu, X.-J. Luo, X.-W. Zhang, J.P. Giesy, B.-X. Mai. 2019. Spatial Distribution and Hazard of Halogenated Flame Retardants and Polychlorinated Biphenyls to Common Kingfisher (Alcedo atthis) from a Region of South China Affected by Electronic Waste Recycling. *Envir. Internat.* 130:104952:1-9.

**22.** Li, C.-C., W.-Y. Feng, H.-Y. Chen, X.-F. Li, F.-H. Song, W.-J. Guo, J.P. Giesy and F.-H. Sun. 2019. Temporal Variation in Zooplankton and Phytoplankton Community Species Composition and the Affecting Factors of Lake Taihu-a Large Freshwater Lake in China. *Environ. Pollut.* 245:1050-1057.

# Curriculum Vitae

**Name/Affiliation:** Yuwei Xie, MEng, Ph.D.

**Current Job Title:** Postdoctoral Fellow, Toxicology Centre, U of S, 2017-Present

## Key Awards and Distinctions

- The best student Paper, the 2nd Korea-China Symposium on Environmental Health & Ecotoxicology, 2016, Seoul, Korea.

## Relationship to Other Grants

**1) Title:** Integration and demonstration of on-site monitoring, diagnosis, and early warning technology for aquatic ecological health in Lake Tai Basin. **Funding Source:** Major Science and Technology Program for Water Pollution Control and Treatment, 2011-2016 **(#2017ZX07602-002)**. **PI:** Xiaowei Zhang. **Amount:** $ 656,932 (CNY: 3,500,000). **Relationship to Current Proposal:** This project supported my Ph.D. program. I developed eDNA-based biomonitoring platform and applied this platform for assessment of the health of the aquatic ecosystem in Lake Tai Basin, Yangtze River, Bohai Sea and Taean coast of South Korea. This project is allowing us to develop, integrate and validate ecogenomics technologies in the current project, but no funds can be transferred to Canada so there is no fiscal overlap with the current proposal.

## Most Significant Contributions

*Development of the next generation of biodiversity and functional assessment of aquatic ecosystem by Ecogenomics*. I initiated the first project in China to develop the next generation ecogenomics technologies to provide high throughput monitoring and assessment of biodiversity in rivers and lakes. In this project, a set of advanced environmental DNA meta-barcoding methods (eDNA) were developed to provide powerful and unbiased tools for fine-scale monitoring of biodiversity in ecosystems. These new technologies have already been adopted by the Jiangsu Provincial program of monitoring aquatic systems, which has started to revolutionize or improve the environmental management practice for the aquatic ecosystems, such as river and lake in China and the rest of world.

## Key Publications

**Peer-reviewed publications: 34; Citations:>315; h-index:12; i10-index:14 (Google Scholar®)**

1. Markwart B., Liber K.*, Xie Y.-W., Raes K., Hecker M., Janz D. and Doig L. E.. Selenium oxyanion bioconcentration in natural freshwater periphyton. Ecotoxicology and Environmental Safety, 30, 2019, 693-704.
2. Li F.-L., Y. Peng, Fang W.-D., Altermatt F., Xie Y.-W., Yang J.-H., and Zhang X.W.*. Application of Environmental DNA Metabarcoding for Predicting Anthropogenic Pollution in Rivers. Environmental Science & Technology 52 (20), 2018, 11708-11719.
3. Zhang X.-H., Tang S. *, Wang M., Sun, W.-M. Xie, Y.-W. and Peng H., A.M. Zhong, H.L. Liu, X.W. Zhang, H.X. Yu, J. P. Giesy, and Markus Hecker*. Acid mine drainage affects the diversity and metal resistance gene profile of sediment bacterial community along a river. Chemosphere, DOI: 10.1016/j.chemosphere.2018.10.210

4. Xie Y.W., Floehr T., Zhang X.-W.*, Xiao H.-X., Yang J.-H., Xia P., Burton G. A. Jr., and Hollert H.. In situ microbiota distinguished primary anthropogenic stressor in freshwater sediments. *Environ. Pollu.*, 239: 189–197 (2018).
5. Xie Y.-W., Zhang X.-W. *, Yang J.-H., Kim S., Hong S., Giesy J.P., Yim U.H., Shimg W.J., Yu H.-X., and Khim J.S. **. eDNA-Based Bioassessment of Coastal Sediments Impacted by an Oil Spill. *Environ. Pollu.*, 238: 739–748 (2018).
6. Yang J.-H., Zhang X.-W. *, Xie Y.-W., Song C., Sun J.-Y., Zhang Y., Giesy J. P. and Yu H.-X.. Ecogenomics of Zooplankton Community Reveals Ecological Threshold of Ammonia Nitrogen. Environ. Sci. Technol., 2017, 51 (5), pp 3057–3064.
7. Xie Y.-W., Wang J.-Z., Yang J.-H., Giesy J. P., Yu H.-X. and Zhang X.-W.*. Environmental DNA metabarcoding reveals primary chemical contaminants in freshwater sediments from different land-use types. *Chemosphere*, 172:201-209 (2017).
8. Xie Y.-W., Zhang X.-W.*, Yang J.-H., Hong S., Kim S., Giesy J. P., Wang T., Lu Y.-L, Yu H.-X. and Khim J.S.. Environmental DNA metasystematic associations with anthropogenic contaminants in sediments from the north coastal areas of the Bohai Sea, China. *Chemosphere*, 172:166-174 (2017).
9. Xie Y.-W., Xia P., Wang H., Yu H.-X., Giesy J. P., Zhang Y.-M., Mora M. A. and Zhang X.-W.*. Effects of captivity and artificial breeding on microbiota in feces of the red-crowned crane (*Grus japonensis*). *Sci. Rep.* **6**, 33350; doi: 10.1038/srep33350 (2016).
10. Xia P., Zhang X.-W.*, Xie Y.-W., Guan M., Villeneuve D. L. and Yu H.-X.. Functional Toxicogenomic Assessment of Triclosan in Human HepG2 Cells Using Genome-Wide CRISPR-Cas9 Screening. *Environ. Sci. Technol.*, 50 (19), 10682–10692 (2016). ***(Cover paper, one of the best science papers of 2016 in ES&T)***
11. Xie Y.-W., Wang J.-Z., Wu Y., Ren C., Song C., Yang J.-H., Yu H.-X, Giesy J. P. and Zhang X.-W. *. Using *in situ* bacterial communities to monitor contaminants in river sediments. *Environ. Pollu*. **212**, 348-357 (2016).

# Curriculum Vitae

**Name/Affiliation:** Markus HECKER, Ph.D., University of Saskatchewan

## Current Job Title

2016-Present Professor (full-time, tenured) and Canada Research Chair, School of the Environment & Sustainability, University of Saskatchewan

## Key Awards and Distinctions

KEY AWARDS: Member of the College of New Scholars, Artists and Scientists of the Royal Society of Canada; 2010 Sabex "Award for Innovation" from the Saskatoon Chamber of Commerce; 2012 CONNECT award from the Alexander von Humboldt Foundation and the US National Academy of Sciences; Erasmus Mundus PROMODOC Ambassador in support of the program to promote doctoral studies in the European Union; Visiting Professor at the Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China; Guest Professor at Xiamen University, Xiamen, China.

DISTINCTION: Prof. Hecker has published over 180 peer reviewed research articles, review articles, editorials and book chapters that have received more than 6700 citations. His Hirsch (H) index is 48 and his *i*10 Index is 116 (Google Scholar®). He has served as an expert for the Chemical Management Plan of the Government of Canada (Environment & Climate Change Canada and Health Canada), US-EPA and the Organization for Economic Coordination and Development's (OECD) Non Animal Validation and Management Testing Group; Dr. Hecker serves as the Editor-in-Chief for Aquatic Toxicology. Furthermore, he is an Editorial Board Member for *Ecotoxicology & Environmental Safety*, and has been a principal editor of *Environmental Science & Pollution Research* between 2007 and 2019. He is also an editor and the North American liaison to *Environmental Sciences Europe*. Dr. Hecker is frequently invited by public and private organizations to give keynote or other invited presentations. One highlight was his role as the introductory speaker at the United States National Academy of Science 17th Annual German-American Kavli Frontiers of Science Symposium (2011). He is a member of the board of the Society for the Advancement of Adverse Outcome Pathways (2014), as well as a board member of the Society of Environmental Toxicology & Chemistry, the largest global society in the field.

## Research Funding

Since commencing his position at the U of S in 2011, Dr. Hecker has received over $16M as the principal or co-principal investigator. Selected examples of recent funding include:

*NSERC, RTI Program:* "Critical Need for a New Chiller for the Aquatic Toxicology Research Facility" (Total Funding - $149,233; 2017-2018).

*NSERC, CRD Program: "*Effects Driven Assessment and Management of Complex Operating Sites" (Total Funding: 1,698,200; 2018-2021).

*Genome Canada, Large-Scale Applied Research Project Competition: Natural Resources and the Environment: Sector Challenges - Genomic Solutions:* "EcoToxChip: A toxicogenonomics tool for chemical prioritization and environmental management" (Total funding: $9,638,754; 2016-2020).

*Canada Research Chair (CRC) Program:* CRC in "Predictive Aquatic Ecotoxicology" (Total Funding 500,000 Can$/5 years; 2016-2021).

*NSERC, Strategic Projects Program*: "Advancing environmental risk assessment of selenium (ERASe)" (Total funding: $725,070; 2015-2018).

*Western Economic Diversification Canada, Saskatoon, SK:* "Analytical toxicology base in support of economic development" (Total funding: $961,000; 2014).

## Most Significant Contributions (references correspond to Key Publications)

*Global Water Futures: Solutions to Water Threats in an Era of Global Change*

Prof. Hecker's research program in Predictive Aquatic Ecotoxicology aims to generate novel information and provide improved strategies and expertise to academic, public, and private sector organizations in support of the protection of Canadian aquatic ecosystems and sustainable management of Canada's resources.

1. ***Application of next generation approaches to advance ecological risk assessment*** - Dr. Hecker's research addresses key challenges in regulatory toxicology and environmental risk assessment. It aims to find practical solutions for the much-needed assessment of an ever-increasing number of chemicals to meet new legislative mandates, while reducing animal use, costs, and time required for testing. The approach chosen aims to organize existing and emerging knowledge to facilitate greater application of data derived through high-throughput *in vitro*, high content omics and imaging, and biomarker approaches, in risk-based decision-making (e.g. *Env. Tox. Chem.* 38(2): 279, 38(6): 1152-1163, & 30:64; *PeerJ Preprints.* 7:e27839v1; *Aq. Tox.* 150:27 & 152:273; *Tox. Sci.* 142:312; *Env. Sci. Tech.* 48:8219 & 50:4826). This research describes stressor pathways and identifies so called "molecular initiating events" that are predictive of biological relevant outcomes in response to the exposure with complex (i.e. oilsands process water, mining and municipal effluents) or specific contaminants of concern (dioxin-like compounds, endocrine disruptors, metals) using next generation omic technologies together with classic toxicological and ecological assessment tools (e.g. *Env. Sci. Tech.* 52(13): 7524–7533, & 47:4822; *Aq. Tox.* 105:218; *Chemosphere* 80:578; *Comp. Bioch. Phys. C* 157:227; *Water Res.* 46:6359 & 47:1545).

2. ***Monitoring the environment for effects of complex mixtures of chemicals*** – Prof. Hecker is involved in the bioanalytical (biological systems) monitoring and the assessment of risks of contaminated aquatic environments. The focus of his research is on effects of so-called endocrine active substances, other emerging contaminants and persistent organic pollutants, and complex mixtures of these compounds on wildlife and humans. His work has significantly contributed to further our understanding of the contamination of a number of global aquatic environments (e.g. *Aq. Tox.* 152:273; *Env. Tox. Chem.* 31:1053; *Env. Sci. Tech.* 45:6268; *PLOS1* 8:e75596; *Tox. Let.* 221:S217; *Water Res.* 47:1545).

3. ***Development of novel and high through-put screening approaches for (environmental) chemicals*** - Examples of technologies Dr. Hecker's group has developed include novel, high-throughput and alternative screening approaches for ocntaminants and complex mixtures such as municipal and industrial effluents (e.g. *Tox. Appl. Pharm.* 217:114; *Aq. Tox.* 152:273). One of the assays, the H295R Steroidogenesis Assay, has been included as a replacement for two existing live animal tests in the US-EPA's Endocrine Disruptor Screening and Testing Program (*Anal. Bioch. Chem.* 390:287-291). Under his guidance, the H295R Steroidogenesis Assay has been validated as part of an Organization for Economic Cooperation and Development (OECD) test method validation program (*Env. Sci. Pol. Res.* 18:503). This test was recently adopted by both the US-EPA and the OECD, and a large number chemicals used in commerce are currently required to be tested with this assay system. He has been granted a patent for an improved H295R Steroidogenesis test system (US Pat. #: US 08501401).

4. ***Understanding the sensitivity of native species of concern to priority pollutants*** – Dr. Hecker's research focuses on cross-species extrapolation of sensitivity to environmental stressors. Specifically, his work studies the sensitivity of species that serve as indicators for ecosystems of interest including endangered and long-lived species such as sturgeons. His studies aim to elucidate the processes that govern species- and lifestage-specific sensitivity to contaminants of concern to enable more realistic and unbiased approaches for ecological risk assessment (e.g. *Hecker 2018, Springer, pp 107-132; Env. Sci. Tech.* 52(13): 7524–7533, & 49:4681; *Aq. Tox.* 114-115:125, 126: 42, 144-145:155, 152:273; *Env. Tox. Chem.* 30:2497 & 22:139).

## Relationship to Other Grants

***Title:*** "Effects Driven Assessment and Management of Complex Operating Sites" ***Source:*** NSERC, 2018-2020 ***Amount:*** $1,698,200 over 4 years ***Summary:*** The aim of this project is to characterize the potential

human health and ecological hazards of groundwater from a contaminated heritage site (pesticide manufacture). There will be no direct financial overlap between this CRD grant and the CFREF project.

***Title:*** "EcoToxChip: A toxicogenonomics tool for chemical prioritization and environmental management" ***Source:*** Genome Canada, 2016-2020 ***Amount:*** $9,638,754 over 4 years ***Summary:*** This project develops, tests and validates quantitative qPCR arrays (EcoToxChips) to address a global need for advanced toxicity testing tools that are accessible, affordable, consistent, and reliable while reducing the need for using live animals under current chemical testing programs. While there will be no direct financial overlap between this Genome Canada and the CRD project, some of the infrastructure, omics' technologies and bioinformatics platforms obtained through the Genome Canada grant will be available to this CFREF project. Furthermore, there is a conceptual relationship between this and the CFREF project.

***Title:*** "Advancing environmental risk assessment of selenium (ERASe)" ***Source:*** NSERC, 2015-2019 ***Amount:*** $725,070 over 3 years ***Summary:*** This project develops an advanced, state-of-the-art risk assessment approach for Se in support of the Canadian resource industry's predictive modeling requirements. Key processes that govern the transformation and assimilation of Se, and its transfer along aquatic food chains is researched. It studies the relative sensitivity and the underlying mechanisms of toxicity of early life stages of native fish species. This will enable prediction of aquatic ecosystem responses of regulatory relevance based on mechanistic toxicity information across diverse fish species (adverse outcome pathways). There will be no direct financial overlap between this and the CFREF project.

***Title:*** "Predictive Aquatic Eco-Toxicology" ***Source:*** Canada Research Chair Program, 2016-2021 ***Amount:*** $500,000 over 5 years ***Summary:*** The CRC will generate novel knowledge and approaches enabling prospective and objective assessments of risks resulting from anthropogenic and natural stressors to Canadian freshwater species. The program will identify stress-initiating events in laboratory models and other fish species, link them with population relevant effects, and use this information to develop predictive models to aid regulators and industry to fulfill their mandates to test chemicals and industrial/muncipal effluents for their safety in a human health and environmental context. While there is a conceptual relationship between the CRC and this project, there will be no overlaps in funding.

***Title:*** "Analytical toxicology base in support of economic development" ***Source:*** Western Economic Diversification, 2014-2017 ***Amount:*** $961,000 once ***Summary:*** This was an equipment grant trough which an Orbitrap Mass spectrometer, and ICP-MS-MS were acquired. The instruments are critical in providing analytical support for this project. There is no overlap in funding with the proposed CFREF project.

***Title:*** "Functional Transcriptomics of Native Canadian Fish Species" ***Source:*** NSERC, 2017-2022 ***Amount:*** $195,000 over 5 years ***Summary:*** This project aims to characterize biological pathways that regulate reproduction in fish native to Canadian freshwater ecosystems, and alterations of which can be used as indicators of potential health risks. The aim of the proposed research is to compare differences in sensitivity of native fishes to environmental stressors of relevance to Canadian water bodies, and to elucidate the molecular basis for these differences. There is some conceptual overlap, but no financial overlap.

**Select Publications (HQP underlined)**

**Peer-reviewed publications: >180; Citations: >6700; h-index: 48; i10-index: 112 (Google Scholar®)**

1. Basu N., Crump D., Head J., Hickey G., Hogan N., Maguire S., Xia J., Hecker M. 2019. EcoToxChip: A next-generation toxicogenomics tool for chemical prioritization and environmental management. *Environ. Toxicol. Chem.* 38(2): 279.
2. Hecker M., LaLone C. 2019. Adverse Outcome Pathways: Moving from a Scientific Concept to an Internationally Accepted Framework. *Environ. Toxicol. Chem.* 38(6): 1152-1163.

3. Zhang X., Tang S., Wang M., Sun W., Xie Y., Peng H., Zhong A., Liu H., Zhang X., Yu H., Giesy J.P., Hecker M. 2019. Acid mine drainage affects the diversity and metal resistance gene profile of sediment bacterial community along a river. *Chemosphere.* 217:790-799.
4. Soufan O., Ewald J., Viau C., Crump D., Hecker M., Basu N., Xia J. 2019. T1000: A reduced toxicogenomics gene set for improved decision making. *PeerJ Preprints.* 7:e27839v1.
5. Tse T.J., Doig L.E., Tang S., Zhang X., Sun W., Wiseman S.B., Feng C.X., Liu H., Giesy J.P., Hecker M., Jones P.D. 2018. Combining high-throughput sequencing of sedaDNA and traditional palaeolimnological techniques to infer historical trends in cyanobacterial communities. *Environ. Sci. Technol.* 52(12): 6842-6853.
6. Doering J.A., Wiseman S.B., Giesy J.P., Hecker M. 2018. A cross-species quantitative adverse outcome pathway for activation of the aryl hydrocarbon receptor leading to early life stage mortality in birds and fishes. *Environ. Sci. Technol.* 52(13): 7524–7533.
7. Hecker M. 2018. Non-Model Species in Ecological Risk Assessment. In: A Systems Biology Approach to Advancing Adverse Outcome Pathways for Risk Assessment. Murphy C. and Reyero N., Eds., Springer, New York, USA. pp 107-132.
8. LaLone C, Ankley GT, Belanger SE, Embry MR, Hodges G, Knapen D, Munn S, Perkins EJ, Rudd MA, Villeneuve DL, Whelan M, Willett C, Zhang X, Hecker M. 2017. Advancing the adverse outcome pathway framework—An international horizon scanning approach. *Environ. Toxicol. Chem.* 36:1411-1421.
9. Sun J., Tang S., Peng H., Saunders D.M.V., Doering J.A., Hecker M., Jones P.D., Giesy J.P., Wiseman S. 2016. A combined transcriptomic and proteomic approach to identify toxicity pathways in early-life stages of Japanese medaka (*Oryzias latipes*) exposed to 1,2,5,6-tetrabromocyclooctane (TBCO). *Environ. Sci. Technol.* DOI: 10.1021/acs.est.6b01249.
10. Doering J.A., Tang S., Peng H., Eisner B.K., Sun J., Giesy J.P., Wiseman S., Hecker M. 2016. High conservation in transcriptomic and proteomic response of white sturgeon to equipotent concentrations of 2, 3, 7, 8-TCDD, PCB 77, and benzo [a] pyrene. *Environ. Sci. Technol.* 50: 4826.
11. Brinkmann M., Koglin S., Eisner B., Wiseman S., Hecker M., Eichbaum K., Thalmann B., Buchinger S., Reifferscheid G., Hollert H. 2016. Characterisation of transcriptional responses to dioxins and dioxin-like contaminants in roach (*Rutilus rutilus*) using whole transcriptome analysis. *Sci. Total Environ.* 541:412.
12. Doering J., Farmahin R., Wiseman S., Beitel S., Kennedy S., Giesy J., Hecker M. 2015. Differences in activation of aryl hydrocarbon receptors of white sturgeon relative to lake sturgeon are predicted by identities of key amino acids in the ligand binding domain. *Environ. Sci. Technol.* 49: 4681.
13. Villeneuve D.L., Crump D., Garcia-Reyero N., Hecker M., Hutchinson T., LaLone C.A., Landesmann B., Lettieri T., Munn S., Nepelska M., Ottinger M.A., Vergauwen L., Whelan M. 2014. Adverse outcome pathway (AOP) development I: Strategies and principles. *Tox. Sci*. 142:312.
14. Beitel S.C., Doering J.A., Patterson S.E., Hecker M. 2014. Assessment of the sensitivity of three North American fish species to disruptors of steroidogenesis using *in vitro* tissue explants. *Aquat. Toxicol.* 152:273.
15. Doering J.A., Wiseman S., Beitel S.C., Giesy J.P., Hecker M. 2014. Identification and expression of aryl hydrocarbon receptors (AhR1and AhR2) provide insight in an evolutionary context regarding sensitivity of white sturgeon (*Acipenser transmontanus*) to dioxin-likecompounds. *Aquat. Toxicol.* 150:27.
16. Maletz S., Floehr T., Beier S., Kluemper C., Brouwer A., Behnisch P., Higley E., Giesy J.P., Hecker M., Gebhardt W., Linnemann V., Pinnekamp J., Hollert H. 2013. *In vitro* characterization of the effectiveness of enhanced sewage treatment processes to eliminate endocrine activity of hospital effluents. *Water Res.* 47: 1545.
17. Vardy D.W., Oellers J., Doering J.A., Hollert H., Giesy J.P., Hecker M. 2013. Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper. *Environ. Toxicol. Chem.* 32:139-147.

# Curriculum Vitae

**Name/Affiliation:** Paul D. JONES, University of Saskatchewan

## Current Job Title

Associate Professor, School of Environment and Sustainability/Toxicology Program 2009-present.

## Key Awards and Distinctions

- Premiers Award for Collaboration (Premier of the Northwest Territories) 2012

## Research Funding

Since 1991 Dr Jones has been the recipient as PI or Co-PI on grants worth over $10 million. This includes grants from federal agencies, provincial/territorial authorities, industry and NGOs.

## Most Significant Contributions

Prof. Jones is recognized internationally as a leading expert on ecotoxicology and the environmental fate of priority and emerging contaminants. He has published extensively on environmental assessment and biomonitoring of multiple stressors associated with a variety of human activities and provided advice to numerous agencies internationally.

***1. Fate and bioaccumulation of contaminants.*** Dr Jones' has studied the environmental fate and effects of persistent organic pollutants for almost three decades. This work has ranged from the ultra-trace analysis of dioxins and PCBs to the analysis of contaminants of emerging concern. Dr Jones has applied a variety of analytical and bioanalytical techniques in the course of these studies ranging from the most advanced forms of analytical mass spectrometry to advances in the use of genomic techniques in environmental toxicology.

***2. Perfluorinated compounds.*** Dr Jones was lead investigator into the biological impacts of perfluorinated chemicals (PFCs) in the early 2000s. As part of the team which first recognized PFCs to be a major environmental contaminant of global concern Dr. Jones directed a team of students and PDFs to investigate the toxic modes of action of these contaminants. Dr Jones has recently received funding from Canada's Department of Fisheries and Oceans to further extend his studies on PFCs in the environment.

***3. Community Based Monitoring*** Dr Jones obtained funding for and lead the SWEEP project which developed community based environmental monitoring practices with First Nations communities in the Slave river and delta. This high profile work funded by the Canadian Water Network (CWN) developed a practices for the incorporation if western science and traditional knowledge into a monitoring framework (Mantyka-Pringle et al 2017). This community approach resulted in a letter of support for the GWF proposal from the Slave River and Delta Partnership.

***4. Infrastructure for Multiple 'Omics Techniques*** Dr Jones lead a proposal to the GWF program which provided $2,200,000 for the purchase of advanced mass spectrometry and DNA sequencing instrumentation to support the goals of the GWF. This effort provides all GWF investigators with access to state of the art instrumentation for the monitoring of the environmental fate and effects of chemical contaminants.

***5. Ancient and Environmental DNA*** Dr Jones had pioneered the use of eDNA technologies on the Canadian prairies. Studies have included the use of eDNA to assess changes in algal communities in Lake Diefenbaker (Tse *et al.* In Press) and the collection of 9,500 year old DNA from the sediments of a small lake in northern Saskatchewan.

## Relationship to Other Grants

Current Funding:

1. **Jones, P.D**., Giesy J.P. and Raine, J. (2017) "Potential Impacts of Modern Perfluorinated Chemicals on Fisheries" Department of Fisheries and Oceans, Canada. Apr 2017 – Mar 2020. $257,600. (Potential exchange of lipidomics samples)
2. Jardine, T., **Jones P.D.** and Giesy, J.P. (2017) "Evaluating effects of the Husky Energy oil spill on fishes in the North Saskatchewan River" Department of Fisheries and Oceans, Canada. Apr 2017 – Mar 2020, $224,250. (Potential exchange of lipidomics samples)
3. **Jones, P.D**. and Giesy, J.P. (2016) "Infrastructure to Support Multiple 'Omics' Analysis". Global Water Futures. $2,200,000. (This GWF funded equipment is essential to this proposal)
4. **Jones, P.D.** and Jardine, T. (2017) "Biological Indicators for the Slave River" Government of the Northwest Territories. $23,000. (No relationship to the current proposal)
5. PI Srinivas Sura**: P.D. Jones** (Co-I)**:** "Assessment of Occurrence of Synthetic Hormone [melengestrol acetate (MGA), trenbolone acetate (TBA)] and Beta-agonist (ractopamine) in Cattle Operations and Associated Environments". Beef Cattle Research council of Canada. 2018-2021. $502,000 CAD. (No relationship to Current Proposal)
6. PI J.P. Giesy: **P. D. Jones** (Co-I): "Next generation solutions to Ensure Healthy Water Resources for Future Generations". Global Waters Future Pillar 3. $1,391,228 CAD, 2016 - 2019. (The current proposal focuses on biochemistry and environmental chemistry, while the previously funded project is focused on Biology. The proposed research is to develop and apply advanced chemistry like proteomics, metabolomics and lipidomics as well as targeted and untargeted chemical analyses while the subject of the other project is genomics (eDNA).

## Key Publications

Prof. Jones has published over 140 peer reviewed journal articles and book chapters, plus technical publications that have received more than 8000 citations (currently >5000/y). His Hirsch (H) index is 47 and his *i*10 Index is 118 (Google Scholar®). Selected examples of key publications are listed below.

Gracia, T., Hilscherova, K., **Jones, P.D.,** Newsted, J.L., Higley, E.B., Zhang, X., Hecker, M., Murphy, M.B., Yu, R.M.K., Lam, P.K.S., Wu, R.S.S., Giesy, J.P. Modulation of steroidogenic gene expression and hormone production of H295R cells by pharmaceuticals and other environmentally active compounds. (2007) Toxicology and Applied Pharmacology, 225 (2), pp. 142-153.

Gracia, T., **Jones, P.D**., Higley, E.B., Hilscherova, K., Newsted, J.L., Murphy, M.B., Chan, A.K.Y., Zhang, X., Hecker, M., Lam, P.K.S., Wu, R.S.S., Giesy, J.P. Modulation of steroidogenesis by coastal waters and sewage effluents of Hong Kong, China, using the H295R assay. (2008) Environmental Science and Pollution Research, 15 (4), pp. 332-343.

Puzyn, T., Falandysz, J., **Jones, P.D**., Giesy, J.P. Quantitative structure - Activity relationships for the prediction of relative in vitro potencies (REPs) for chloronaphthalenes. (2007) Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering, 42 (5), pp. 573-590.

Jordaan, I., Pieters, R., Quinn, L.P., Giesy, J.P., **Jones, P.D**., Murphy, M.B., Bouwman, H. The contribution of dioxin-like compounds from platinum mining and processing samples. (2007) Minerals Engineering, 20 (2), pp. 191-193.

Gracia, T., Hilscherova, K., **Jones, P.D**., Newsted, J.L., Zhang, X., Hecker, M., Higley, E.B., Sanderson, J.T., Yu, R.M.K., Wu, R.S.S., Giesy, J.P. The H295R system for evaluation of endocrine-disrupting effects. (2006) Ecotoxicology and Environmental Safety, 65 (3), pp. 293-305.

Newsted, J.L., **Jones, P.D.,** Coady, K., Giesy, J.P. Avian toxicity reference values for perfluorooctane sulfonate. (2005) Environmental Science and Technology, 39 (23), pp. 9357-9362.

**Jones, P.D.,** Newsted, J.L., Henningsen, G., Slocomb, J., Giesy, J.P. Distribution of PCDDs and PCDFs in soils collected from the Denver front range: Principal components analysis of diffuse dioxin sources. (2005) Environmental Science and Pollution Research, 12 (4), pp. 189-198.

Young, A.L., Giesy, J.P., **Jones, P.D**., Newton, M. Environmental fate and bioavailability of agent orange and its associated dioxin during the Vietnam War. (2004) Environmental Science and Pollution Research, 11 (6), pp. 359-370.

Hilscherova, K., **Jones, P.D.,** Gracia, T., Newsted, J.L., Zhang, X., Sanderson, J.T., Yu, R.M.K., Wu, R.S.S., Giesy, J.P. Assessment of the effects of chemicals on the expression of ten steroidogenic genes in the H295R cell line using real-time PCR. (2004) Toxicological Sciences, 81 (1), pp. 78-89.

**Jones, P.D.,** Hu, W., De Coen, W., Newsted, J.L., Giesy, J.P. Binding of perfluorinated fatty acids to serum proteins. (2003) Environmental Toxicology and Chemistry, 22 (11), pp. 2639-2649.

Ohiozebau, E., Tendler, B., Codling, G., Kelly, E., Giesy, J.P., **Jones, P.D.** Potential health risks posed by polycyclic aromatic hydrocarbons in muscle tissues of fishes from the Athabasca and Slave Rivers, Canada. (2017) Environmental Geochemistry and Health, 39 (1), pp. 139-160.

Mantyka-Pringle, C.S., Jardine, T.D., Bradford, L., Bharadwaj, L., Kythreotis, A.P., Fresque-Baxter, J., Kelly, E., Somers, G., Doig, L.E., **Jones, P.D**., Lindenschmidt, K.-E., Bridging science and traditional knowledge to assess cumulative impacts of stressors on ecosystem health, Environment International, Volume 102, May 2017, pp. 125-137.

Peng, H., Chen, C., Cantin, J., Saunders, D.M.V., Sun, J., Tang, S., Codling, G., Hecker, M., Wiseman, S., **Jones, P.D.,** Li, A., Rockne, K.J., Sturchio, N.C., Cai, M., Giesy, J.P. Untargeted Screening and Distribution of Organo-Iodine Compounds in Sediments from Lake Michigan and the Arctic Ocean. (2016) Environmental Science and Technology, 50 (18), pp. 10097-10105.

Peng, H., Sun, J., Alharbi, H.A., **Jones, P.D.,** Giesy, J.P., Wiseman, S. Peroxisome Proliferator-Activated Receptor γ is a Sensitive Target for Oil Sands Process-Affected Water: Effects on Adipogenesis and Identification of Ligands. (2016) Environmental Science and Technology, 50 (14), pp. 7816-7824.

Eichbaum, K., Brinkmann, M., Nuesser, L., Buchinger, S., Reifferscheid, G., Codling, G., **Jones, P.,** Giesy, J.P., Hecker, M., Hollert, H. Bioanalytical and instrumental screening of the uptake of sediment-borne, dioxin-like compounds in roach (*Rutilus rutilus*). (2016) Environmental Science and Pollution Research, 23 (12), pp. 12060-12074..

Ohiozebau, E., Tendler, B., Hill, A., Codling, G., Kelly, E., Giesy, J.P., **Jones, P.D**. Products of biotransformation of polycyclic aromatic hydrocarbons in fishes of the Athabasca/Slave river system, Canada. (2016) Environmental Geochemistry and Health, 38 (2), pp. 577-591.

Peng, H., Chen, C., Cantin, J., Saunders, D.M.V., Sun, J., Tang, S., Codling, G., Hecker, M., Wiseman, S., **Jones, P.D**., Li, A., Rockne, K.J., Sturchio, N.C., Giesy, J.P. Untargeted Screening and Distribution of Organo-Bromine Compounds in Sediments of Lake Michigan. (2016) Environmental Science and Technology, 50 (1), pp. 321-330.

*Global Water Futures: Solutions to Water Threats in an Era of Global Change*

Peng, H., Chen, C., Saunders, D.M.V., Sun, J., Tang, S., Codling, G., Hecker, M., Wiseman, S., **Jones, P.D**., Li, A., Rockne, K.J., Giesy, J.P. Untargeted Identification of Organo-Bromine Compounds in Lake Sediments by Ultrahigh-Resolution Mass Spectrometry with the Data-Independent Precursor Isolation and Characteristic Fragment Method. (2015) Analytical Chemistry, 87 (20), pp. 10237-10246.

Peng, H., Saunders, D.M.V., Sun, J., Codling, G., Wiseman, S., **Jones, P.D**., Giesy, J.P. Detection, identification, and quantification of hydroxylated bis(2-ethylhexyl)-tetrabromophthalate isomers in house dust. (2015) Environmental Science and Technology, 49 (5), pp. 2999-3006.

Hong, S., Khim, J.S., Wang, T., Naile, J.E., Park, J., Kwon, B.-O., Song, S.J., Ryu, J., Codling, G., **Jones, P.D.**, Lu, Y., Giesy, J.P. Bioaccumulation characteristics of perfluoroalkyl acids (PFAAs) in coastal organisms from the west coast of South Korea. (2015) Chemosphere, 129, pp. 157-163.

Tse, T.J., Doig, L.E., Leavitt, P.R., Quiñones-Rivera, Z.J., Codling, G., Lucas, B.T., Liber, K., Giesy, J.P., Wheater, H., **Jones, P.D**. Long-term spatial trends in sedimentary algal pigments in a narrow river-valley reservoir, Lake Diefenbaker, Canada. (2015) Journal of Great Lakes Research, 41, pp. 56-66.

Hong, S., Khim, J.S., Park, J., Kim, S., Lee, S., Choi, K., Kim, C.-S., Choi, S.-D., Park, J., Ryu, J., **Jones, P.D**., Giesy, J.P. Instrumental and bioanalytical measures of dioxin-like compounds and activities in sediments of the Pohang Area, Korea. (2014) Science of the Total Environment, 470-471, pp. 1517-1525.

Tse, T.J., Codling, G., **Jones, P.D.,** Thoms, K., Liber, K., Giesy, J.P., Wheater, H., Doig, L.E. Reconstructing long-term trends in municipal sewage discharge into a small lake in northern Manitoba, Canada. (2014) Chemosphere, 103, pp. 299-305.

Codling, G., Vogt, A., **Jones, P.D.,** Wang, T., Wang, P., Lu, Y.-L., Corcoran, M., Bonina, S., Li, A., Sturchio, N.C., Rockne, K.J., Ji, K., Khim, J.-S., Naile, J.E., Giesy, J.P. Historical trends of inorganic and organic fluorine in sediments of Lake Michigan. (2014) Chemosphere, 114, pp. 203-209.

**Jones, P.D.**, Hecker, M., Wiseman, S., Giesy, J.P. Birds. (2013) Endocrine Disrupters: Hazard Testing and Assessment Methods, pp. 272-303.

Wiseman, S.B., He, Y., Gamal-El Din, M., Martin, J.W., **Jones, P.D.,** Hecker, M., Giesy, J.P. Transcriptional responses of male fathead minnows exposed to oil sands process-affected water. (2013) Comparative Biochemistry and Physiology - C Toxicology and Pharmacology, 157 (2), pp. 227-235.

Naile, J.E., Khim, J.S., Hong, S., Park, J., Kwon, B.-O., Ryu, J.S., Hwang, J.H., **Jones, P.D**., Giesy, J.P. Distributions and bioconcentration characteristics of perfluorinated compounds in environmental samples collected from the west coast of Korea. (2013) Chemosphere, 90 (2), pp. 387-394.

Zhang, K., Wan, Y., **Jones, P.D.**, Wiseman, S., Giesy, J.P., Hu, J. Occurrences and fates of hydroxylated polybrominated diphenyl ethers in marine sediments in relation to trophodynamics. (2012) Environmental Science and Technology, 46 (4), pp. 2148-2155.

Peng, H., Saunders, D. M., Sun, J., **Jones, P. D.,** Wong, C. K., Liu, H., & Giesy, J. P. (2016). Mutagenic Azo Dyes, Rather Than Flame Retardants, Are the Predominant Brominated Compounds in House Dust. *Environmental Science & Technology*, *50*(23).

Sun, J., Peng, H., Alharbi, H. A., Jones, P. D., Giesy, J. P., & Wiseman, S. B. (2017). Identification of Chemicals that Cause Oxidative Stress in Oil Sands Process-Affected Water. *Environmental Science and Technology*, *51*(15), 8773–8781.

# Curriculum Vitae

**Name/Affiliation:** Barbara A. KATZENBACK, Ph.D., University of Waterloo

## Current Job Title

2016-Present Assistant Professor (full-time, tenure-track), Dept. of Biology, University of Waterloo

## Key Awards and Distinctions

- 2017 Outstanding Performance Award (University of Waterloo)
- 2014 NSERC Banting Post Doctoral Fellowship
- 2012 NSERC Post Doctoral Fellowship
- 2012 Faculty of Science Doctoral Dissertation Award, University of Alberta
- 2011 Andrew Stewart Memorial Graduate Award, University of Alberta
- 2008 NSERC Alexander Graham Bell Canadian Graduate Scholarship for PhD students
- 2008 Alberta Ingenuity PhD Scholarship
- Published 32 peer reviewed journal articles and book chapters, which have received over 530 citations, with a Hirsch (H) index of 15 and an i10 Index of 19 (Google Scholar®).
- Dissemination of research through presentations at national and international conferences (Total: 67 conference proceedings, 10 invited talks)
- Recognized as a leader in my field; currently serving on the Editorial Board for Comparative Biochemistry and Physiology, have acted as an *Ad hoc* reviewer for national granting agencies and for various immunology and physiology journals.
- Leadership roles in regional and national societies, including being elected the Vice Chair (2016-2017) and Chair (2017-2018) of the Parasitism, Immunity and Environment Section of the Canadian Society of Zoologists.

## Research Funding

As an early career researcher, I have secured funding to equip my laboratory with cutting edge molecular and cellular instrumentation and operating funds to support salaries and research supplies.

Current funding:

1. Flow cytometer for analyses of host-pathogen interactions, NSERC Research Tools and Instruments Grant, 2019-2020, $97,685 (sole PI).
2. Amphibian innate immunity and impact of environmental stress, NSERC Discovery Grant, 2017-2022, $150,000 (sole PI).
3. Next generation solutions to ensure healthy water resources for future generations, Canada First Research Excellence Fund-Global Water Futures, 2017-2020, $1,391,228 (Co-I).
4. Laboratory Start Up Funds, University of Waterloo, 2016-2021, $200,000 (sole PI).

**Most Significant Contributions** (references[x] correspond to Key Publications)

I have established an integrative and innovative aquatic vertebrate environmental immunology research program using an array of techniques that span whole animal experimentation, cellular biology, molecular biology, and translation of these techniques to current issues in environment and toxicology. I have made substantial developments towards understanding innate immunity in ectothermic vertebrates, evolution of this system across vertebrates and the impact of changing environments on animal health and disease susceptibility.

**1. Environmental modulation of aquatic vertebrate host defenses.** This research has advanced our understanding of how changes in an organism's abiotic environment impacts amphibian[7,9] and fish[1,3] host defense. For example, we have shown acute exposure to freezing (extreme cold) resulted in modulation of key immune molecules (antimicrobial peptides) in frogs[9] and low temperature exposure negatively impactes the antiviral response of fish cells[1], ultimately affecting the animal's ability to fend against pathogen insult. These are novel finding as the mechanisms underlying temperature regulated changes aquatic vertebrate susceptibility to pathogens are largely unknown. Examining how environmental stressors impact immunocompetency of vertebrates will be essential in devising predictive tools for use in assessing aquatic ecosystem health.

**2. Development of *in vitro* systems for assessment of aquatic vertebrate immune system function.** I developed a novel *in vitro* system to examine the function of teleost neutrophils, a key innate immune cell[14]. This model system provided important information on fish neutrophil cell biology and neutrophil responses to virulent bacteria that cause mortality in a wide range of teleost species[4,14]. The utility of the *in vitro* neutrophil system and massing insight on the essential role of neutrophils in teleost innate immunity set the stage for applied collaborative studies in toxicology to examine the effects of nanoparticles on neutrophils[6]. This model system has broad application to a number of topics (neutrophil-pathogen interactions, neutrophil inflammatory response, environmental toxicology) resulting in significant advancements towards understanding environmental and anthropogenic factors that contribute to disease susceptibility in fishes.

**3. Transcription factors as fingerprints of immune cell populations.** These studies innovated the use of transcription factor (TF) mRNA levels as a means of identifying and characterizing innate immune cell populations. Using the TF fingerprint, I was able to examine the distribution of progenitor cells in tissues and how progenitor cells responded to cytokines and pathogens[13]. These results were instrumental in providing insight into the intracellular mechanisms of fish innate immune cell development and response to biotic stressors. The TF fingerprint was utilized as a tool for subsequent manuscripts and collaborations to demonstrated how goldfish progenitor cells modulate their transcription factors as they differentiate into various innate immune cell lineages[11]. These tools can be applied to the development of high throughput technologies (eDNA) for creating environmental fingerprints of the organisms, pathogens, and their abundance and will provide insight into population and ecosystem health.

**4. Host-pathogen associations:** Fish are particularly susceptible to pathogens in crowded conditions and during environmental stress, necessitating a detailed understanding of host response to pathogen challenge for the development of effective prevention strategies. As the first line of defense against pathogens, the development and functional activation of innate immune cells is of upmost importance. These studies established the hematopoietic response of fishes to challenge with pathogens (using transcription factor fingerprints) and also demonstrated the critical role fish macrophages and neutrophils play in mediating pro-inflammatory responses and resolution of infection through the deployment of antimicrobial arsenal[4,10,12-14]. This type of experimentation will be required in order to assess immunocompetency of aquatic vertebrates in ecosystems.

## Relationship to Other Grants

I have secured funding for acquisition of cutting-edge research infrastructure and thus there are no available funds to cover the operational costs associated with the current proposed research.

**1. Title:** Flow cytometer for analyses of host-pathogen interactions**. Funding Source:** NSERC Research Tools and Instruments program. **PI:** B.A. Katzenback. **Amount:** $97,685. **Relationship to Current Proposal:** These funds are for the purchase of a flow cytometer that will be used to analyze pathogen-challenged cell populations from various samples. The NSERC RTI funds will be used solely to purchase the flow cytometer and thus do not overlap with the requested funds in the current proposal.

**2. Title:** Amphibian innate immunity and impact of environmental stress**. Funding Source:** NSERC Research Discovery Grant. **PI:** BA Katzenback. **Amount:** $150,000. **Relationship to Current Proposal:** This operating grant supports HQP salaries and operating costs associated with research output. The focus of this grant is to elucidate how secretory molecules on the skin may be contributing to modulating skin immunity in frogs, their susceptibility to a virulent virus (frog virus 3) associated with mass amphibian die offs and how changing environmental temperatures might contribute to the outcome of the host survival. As these are separate research programs, these funds do not overlap with the requested CFREF funds. However, research output from either project may inform or enhance the contextual knowledge of the other and has the potential to be synergistic, providing a whole systems view (molecular to community).

**3. Title:** Next generation solutions to ensure healthy water resources for future generations. **Funding Source:** Canada First Research Excellence Fund-Global Water Futures; 2017-2020. **PI:** JP Giesy. **Amount:** $1,391,228 (Co-I, $133,295 my portion). **Relationship to Current Proposal:** These funds supported the initial phase (Y1-3) of the current proposal (Y4-7). The focus was to develop and validate eDNA metabarcoding tools for biomonitoring of fish and amphibian populations in the Grand River Watershed in partnership with provincial and regional conservation authorities. In conjunction with the development of eDNA metabarcoding tools, sample workflows have been optimized to eliminate environmental inhibitors while maintaining eDNA integrity. Bioinformatics pipelines and sequence classifiers have been established and undergone pilot testing. Field testing has been conducted in collaboration with one of our end users, *rare* Charitable Research Reserve, for fish biodiversity (Baumann Creek) and amphibian biodiversity (various vernal pools) to compare traditional survey methods with next generation eDNA metabarcoding community analysis. The eDNA metabarcoding tools generated during the initial phase of the research project will directly support the proposed research and end user engagement in the current proposal.

**4. Title:** Laboratory Start-Up Funds. **Funding Source:** University of Waterloo Biology Department; April 1, 2016 –March 31, 2021. **PI:** BA Katzenback. **Amount:** $200,000. **Relationship to Current Proposal:** These funds were used to purchase and maintain the following molecular and cellular infrastructure: an Applied Biosystems QS5 quantitative PCR machine, two gradient thermocyclers, a gel electrophoresis apparatus, a Labconco Level II biosafety cabinet, a Millipore water purification system, cell-culture package refrigerated centrifuge, ultralow temperature freezer, cell counter and a number of basic, yet essential, infrastructure items necessary to conducting experimentation including pipettes, scales, a pH meter, stirrers, heating blocks, refrigerated centrifuges, microcentrifuges, vortexes, etc. Funds from my Start-Up grant do not overlap with the requested funds in the current CFREF grant. The support obtained through the proposed CFREF However, this equipment can be used to support the proposed research and training of highly qualified personnel (HQP) in the current proposal in an array of cutting-edge techniques.

## Key Publications

**Peer-reviewed publications: 32; Citations: >530; h-index: 15; i10-index: 19 (Google Scholar®)**

Underlined authors are trainees I have supervised, asterisks indicate authors contributed equally. *IF = impact factor, CS = cite score where IF is not available.*

1. Abram QH, Rodriguez-Ramos T, Bols NC, **Katzenback BA,** Dixon B. 2019. Effect of suboptimal temperature on the regulation of endogenous antigen presentation in a rainbow trout hypodermal fibroblast cell line. *Dev Comp Immunol. In Press. IF 3.119*
2. Varga JFA, Bui-Marinos MP, **Katzenback BA**. 2019. Frog skin innate immune defences: sensing and surviving pathogens. *Front Immunol* 9:3128. *IF6.429*
3. Abram QH**,** Dixon B, **Katzenback BA**. 2017. Impacts of low temperature on the teleost immune system. *Biology* 6:39. *CS 4.42*
4. Hodgkinson JW, Ge JQ, **Katzenback BA**, Havixbeck JJ, Barreda DR, Stafford JL, Belosevic M. 2015. Development of an *in vitro* model system to study the interaction between goldfish (*Carassius auratus* L.) neutrophils and *Mycobacterium marinum*. *Dev Comp Immunol* 53:349-357. *IF 3.119*
5. Pietsch C, **Katzenback BA**, Garcia-Garcia E, Schulz C, Belosevic M, Burkhardt-Holm P. 2015. Acute and subchronic effects on immune responses of carp (*Cyprinus carpio* L.) after exposure to deoxynivalenol (DON) in feed. *Mycotoxin Res* 31: 151-164. *IF 3.741*
6. Ortega VA*, **Katzenback BA***, Stafford JL, Belosevic M, Goss GG. 2015. Effects of polymer-coated metal oxide nanoparticles on goldfish (*Carassius auratus* L.) neutrophil viability and function. *Nanotoxicology* 9:23-33. *IF 7.931*
7. Dawson NJ, **Katzenback BA,** Storey KB. 2015. Free-radical first responders: The characterization of CuZnSOD and MnSOD regulation during freezing of the freeze-tolerant North American wood frog, *Rana sylvatica. Biochimica et Biophysica Acta – General Subjects* 1850:97-106. *IF 3.67*
8. **Katzenback BA***, Dawson NJ*, Storey KB. 2014. Purification and characterization of a urea sensitive lactate dehydrogenase from the liver of the African clawed frog, *Xenopus laevis*. *J Comp Physiol Part B* 184: 601-611. *IF 2.341*
9. **Katzenback BA**, Holden HA, Falardeau J, Childers C, Hadj-Moussa H, Avis T, Storey KB. 2014. Regulation of the *Rana sylvatica* brevinin-1SY antimicrobial peptide during development and in dorsal and ventral skin in response to freezing, anoxia, and dehydration. *J Exp Biol* 217:1392-1401. *IF 3.017*
10. Hagen MO, **Katzenback BA**, Shahinoor MD, Mitchell S, Gamal El-Din M, Belosevic M. 2014. The analysis of immune response after acute and sub-chronic exposures of goldfish (*Carassius auratus* L.) to oil sands process affected water. *Toxicological Sciences* 138: 59-68. *IF 4.081*
11. **Katzenback BA**, Foroutanpay B, Belosevic M. 2013. Expression of transcription factors in goldfish (*Carassius auratus* L.) macrophages and their progenitors. *Dev Comp Immunol* 41: 230-239. *IF: 3.119*
12. Xie J, Hodgkinson JW, **Katzenback BA**, Kovacevic N, Belosevic M. 2013. Characterization of three Nod-like receptors of goldfish (*Carassius auratus* L.) and their role in antimicrobial responses of macrophages to *Aeromonas salmonicida* and *Mycobacterium marinum*. *Dev Comp Immunol* 39: 180-187. *IF: 3.119*
13. **Katzenback BA,** Karpman M, Belosevic M. 2011. Distribution and expression analysis of transcription factors in tissues and progenitor cell populations of the goldfish (*Carassius auratus* L.) in response to growth factors and pathogens. *Mol Immunol* 48: 1224-1235. *IF 3.064*
14. **Katzenback BA,** Belosevic M. 2009. Isolation and functional characterization of neutrophil-like cells from goldfish (*Carassius auratus* L.) kidney. *Dev Comp Immunol* 33: 601-611. *IF: 3.119*

# Curriculum Vitae

**Name/Affiliation:** Bram F. NOBLE, Ph.D., University of Saskatchewan

## Current Job Title

Professor, Department of Geography & Planning

## Key Awards and Distinctions

- International Atomic Energy Agency Expert Panel on Strategic Assessment (2019)
- Canadian Council of Academies Expert Panel Appointment (2016)
- Elsevier's Top 5 Highest Cited Paper in *Environmental Impact Assessment Review* (2014-2016)
- International Association for Impact Assessment 'Best Paper' Award for *Impact Assessment and Project Appraisal* journal (2016)
- Auditor General of British Columbia, Subject Matter Expert on Cumulative Effects (2014-2015)
- Auditor General of Ontario, Expert Advisor on Environmental Assessment (2015-2016)
- National Energy Board Participant Funding Committee (2014-2016)
- Associate Editor, *Journal of Environmental Assessment Policy and Management* and *Environmental Management* (2015 – 2017)
- Great Sand Hills Scientific Advisory Committee, appointed by Saskatchewan Minister of the Environment (2007-2009)
- Auditor General of Canada, Commissioner of Environment and Sustainable Development, Expert Advisory Panel (2003)
- Governor General of Canada Gold Medal (2002)
- NSERC-SSHRC-CIHR Canadian Policy Research Awards Prize (2000)
- Author of Canadian Council of Ministers of the Environment guidelines for regional strategic environmental assessment
- Author of Ministerial decision guidance for federal environmental assessment decisions under the Nunavut Land Claims Agreement
- Co-author of federal technical guidance for assessing cumulative environmental effects under the *Canadian Environmental Assessment Act, 2012*
- Expert witness on cumulative effects for regulatory hearings and legal challenges: Keeyask hydro, Bipole III transmission line; Spectra LNG pipeline; Bruce Power nuclear fuel waste management

## Research Funding

My primary source of funding as principal investigator (> 60%) is SSHRC (Social Sciences and Humanities Research Council of Canada), supplemented by industry and government

- $18.5 million total funding secured as principal *and* co-investigator, including a recent SSHRC Partnership Grant as principal investigator ($8.8 million)

## Relationship to Other Grants

**1) Title:** Community appropriate sustainable energy security. **Funding Source:** Social Sciences and Humanities Research Council of Canada. **PI**: B Noble. **Amount:** $2,500,000. **Relationship to Current Proposal**: This project is focused on energy transition in northern and Indigenous communities. There is no overlap with the current GWF funding request.

**2) Title:** A water quality modelling system of the Qu'Appelle River catchment for long-term water management policy development **Funding Source:** Environment Canada **PI:** K. Lindenschmidt. **Amount:** $309,000 **Relationship to Current Proposal:** My share of funds under this grant are used primarily to support a post-doctoral fellow to assist with the development of a systems dynamic model for the lower Qu'Appelle watershed, and for stakeholder engagement in assessing better management practices for managing land use impacts (drainage, cattle distribution) to water quality. Funds do not overlap with the current GWF funding request.

**3) Title:** Flood risk mitigation in rural communities experiencing rapid environmental change – evaluation of alternative flood policy and mitigation strategies **Funding Source:** SSHRC - Social Sciences and Humanities Research Council of Canada **PI:** B. Noble. **Amount:** $74,108 **Relationship to Current Proposal:** Funds under this grant support a policy analysis of flood risk management policy in the prairie region, and the development of a multi-criteria model for assessing flood risk options. Funds do not overlap with the current GWF funding request.

**4) Title:** NSERC Create for Water Security **Funding Source:** NSERC – Natural Sciences and Engineering Research Council of Canada **PI:** C. Westbrook. **Amount:** $1,650,000 **Relationship to Current Proposal:** All of the funds under this grant are used to support HQP training in the broad area of water security. I have access to a portion of the funds specifically to support HQP training in the social sciences. Funds under NSERC Create do not overlap with the GWF request; however, there is an opportunity to request 'top-up' funds for HQP training through the NSERC Create program.

**5) Title:** Uncertainty analysis and communication in environmental impact assessment practice and decision making **Funding Source:** SSHRC - Social Sciences and Humanities Research Council of Canada **PI:** B. Noble. **Amount:** $292,000 **Relationship to Current Proposal:** This project is focused on how practitioners, project proponents and regulators identify, understand, communicate and consider risk during environmental impact assessment and decision making processes. Funds do not overlap with the current GWF funding request.

**6) Title:** Assessing regulators' information needs to make decision regarding cumulative effects under the Mackenzie Valley Resource Management Act **Funding Source:** GNWT – Cumulative Impact Monitoring Program. **PI:** B. Noble. **Amount:** $148,452 **Relationship to Current Proposal:** This project examines the information needs of regulatory decision makers to make decisions regarding cumulative effects under the Mackenzie Valley Resource Management Act, and assesses whether current aquatic monitoring programs provide the information required to meet decision support needs. Funds do not overlap with the current GWF funding request. However, the approach and questions explored in this project (regulatory / decision maker needs) are likely applicable to the policy and community aspects of the proposed GWF project.

## Most Significant Contributions

My research is focused on various aspects of impact assessment. I have been involved in research and professional practice related to resource development, impact assessment, regulatory processes, and the energy sector for 18 years. Since completing my PhD in 2002, I have published 90 peer reviewed journal papers (*h-index* = 33); 23 book chapters; authored a leading textbook on impact assessment (currently in its 3rd edition); produced more than 50 policy and technical reports for industry, government agencies, public thinktanks, and Indigenous governments; and have given move than 50 invited national and international keynote presentations, and 85 conference and workshop papers. I am shaping the field of impact assessment through the development of widely used good-practice guides, and am a sought-out expert by industry, governments and First Nations for guidance on impact assessment, resource policy, and regulatory/legal challenges.

- My research on strategic environmental assessment (SEA) established the principles and methodological framework that shaped how the tool and practice has evolved, in Canada and internationally. I co-led the first regional SEA in Canada, which is one of the most referenced test cases; developed national SEA guidelines for the CCME; co-authored the south Athabasca oil sands regional SEA; and advised on the 2014 Chilean national assessment of its electricity sector.
- My research on cumulative effects assessment (CEA) established institutional and governance principles, as well as basic science requisites, for 'good practice' CEA. This work set out fundamental principles that were used to inform a provincial audit of regulatory practice in BC and was the primary input for the development of a CEA and management framework for the Elk Valley, co-led by Teck Coal, BC, and the Ktunaxa First Nation. My work has been used as evidence in a number of regulatory challenges over project developments and appeal board hearings, including the Keeyask hydroelectric project and Bipole III project (MB), and a Fort Nelson First Nation legal challenge over a Nexen water license for hydraulic fracturing.
- Much of this work on impact assessment regulations has focused on advancing assessment practices for Aboriginal peoples. This includes a recent series of public policy reports commissioned by the Macdonald Laurier Institute, an independent national public policy think tank, focused on reforming environmental assessment across Canada to better engage Aboriginal peoples, and to honor the principles as set out under the United Nations Declaration on the Rights of Indigenous Peoples.

## Key Publications

**Select peer-reviewed journal publications:** h-index: 33 (Google Scholar®)

**1.** Morrison A, Noble BF, Westbrook C. 2019. Flood risk management in Canada's Prairie provinces: An analysis of decision-maker priorities and policy preferences. *Environ Manage*. Accepted 31 Aug
**2.** Noble BF 2019. Transforming IA from the outside in: capacity and levers for strategic assessment. *Impact Assess Proj Apprais* Accepted 29 Aug 2019
**3.** Arnold L, Hanna K, **Noble B.** 2019. Freshwater cumulative effects and environmental assessment in the Mackenzie Valley, Northwest Territories: challenges and decision-maker needs. *Impact Assess Proj Apprais.* https://doi.org/10.1080/14615517.2019.1596596

*Global Water Futures: Solutions to Water Threats in an Era of Global Change*

4. **Noble BF**, Gibson R, White L, Blakley J, *Nwanekezie K, Croal P. 2019. Effectiveness of strategic environmental assessment in Canada under directive-based and informal practice. *Impact Assess Proj Apprais* 37(3-4): 344-55.
5. Lindemschmidt et al. 2019. Incorporating social dimensions in hydrological and water-quality modeling to evaluate the effectiveness of agricultural beneficial management practices in a Prairie river basin. *Environmental Science and Pollution Research*. Accepted for publication.
6. Hanna K, McGuigan E, **Noble BF**, Parkins J. 2019. Analysis of the state of impact assessment research for low carbon power production. Energy Res Soc Sci 50: 116-128
7. Crowley C, Blakley J, Jaeger J, **Noble BF**, Westman C. 2018. Improving uncertainty disclosure and communication in environmental assessment: Lessons from an energy development case in northern Alberta. *J Environ Plan Manage*. doi.org/10.1080/09640568.2019.1579973
8. Hassanzadeh E, Strickert G, Morales-Marin L, **Noble BF**, Baulch H, Shupena-Soulodre E, Lindenschmidt K. 2019. A framework for engaging stakeholders in water quality modeling and management: Application to the Qu'Appelle River Basin, Canada. *J Environ Manage* 231: 1117-1126
9. Luke L, **Noble BF**. 2018. Consideration of climate change in environmental assessment (EA): An analysis of the liquefied natural gas sector, British Columbia. *Impact Assess Proj Apprais*. https://doi.org/10.1080/14615517.2018.1533515
10. Dibo A, **Noble BF**, Sanchez L. 2018. Perspectives on driving changes in project-based cumulative effects assessment for biodiversity: lessons from the Canadian experience. *Environ Manage*. 62(5):929-941
11. Morrison A, **Noble BF**, Westbrook CJ. 2018. Flood risk management in the Canadian Prairie provinces: Defaulting toward flood resistance and recovery versus resilience. *Cdn Wat Res J* 43(1): 33-46.
12. Cronmiller J, **Noble BF**. 2018. The discontinuity of cumulative effects monitoring in the Lower Athabasca region of Alberta, Canada: Institutional challenges to long-term monitoring and cumulative effects management. *Environ Reviews* 26(2): 169-180
13. Cronmiller J, **Noble BF**. 2018. Integrating environmental monitoring with cumulative effects management and decision making. *Integr Envon Assess Manage* 14(3): 407-417
14. Pavlyuk O,**Noble BF**, Blakley J, Jaeger J. 2017. Fragmentary provisions for uncertainty disclosure and consideration in EA legislation, regulations and guidelines and the need for improvement. *Environ Impact Assess Rev* 66: 14-23
15. Morrison A, Westbrook CJ, **Noble BF**. 2017. A review of the flood risk management governance and resilience literature. *J Flood Risk Manage* doi:10.1111/jfr3.12315.
16. **Noble BF**, Liu G, Hackett P. 2017. The contribution of project environmental assessment to assessing and managing cumulative effects: individually and collectively insignificant? *Environ Manage* 59(4): 531-545
17. Udofia A, **Noble BF**, Poelzer G. 2017. Meaningful and efficient? Exploring the challenges to Aboriginal participation in environmental impact assessment. *Environ Impact Assess Rev* doi.org/10.1016/j.eiar.2016.04.008.
18. Leung W, **Noble BF**, Jaeger J, Gunn J. 2016. Disparate perceptions about uncertainty consideration and disclosure practices in environmental assessment and opportunities for improvement. *Environ Impact Assess Rev* 57: 89-100.
19. Lees J, Jaeger J, Gunn J, **Noble BF**. 2016. Analysis of uncertainty consideration in environmental assessment: an empirical study of Canadian EA practice. *J Environ Plan Manage* 59(1): 2024-2044.

## Curriculum Vitae

**Name/Affiliation:** Vince P. Palace, Ph.D.

**Current Job Title:** Head Research Scientist, IISD-Experimental Lakes Area, 2016-Present

### Selected Awards and Distinctions

| | |
|---|---|
| 2015 | Invited Plenary Speaker, Society of environmental Toxicology and Chemistry, Prairie and Northern Chapter ("The Role of Social License in Environmental Permitting") |
| 2016 | Managing Editor, Archives of Environmental Contamination and Toxicology |
| 2015 | Invited Session Chair, Canadian Ecotoxicity Workshop, Saskatoon, SK |
| 2014 | Invited Session Chair, Canadian Ecotoxicity Workshop, Ottawa ON |
| 2013 | Member of the University of Manitoba search committee CRC Research Chair |
| 2012 | Co-Organizer 13th Workshop on Brominated and Other Flame Retardants, Winnipeg, Canada June 4-5. |
| 2011 | Invited Session chair, SETAC Europe Milan Italy May 15-19 2011. |
| 2010 | Assistant Deputy Minister Distinction Award for contributing to the Stockholm Convention's decision to initiate global action on the flame retardant Hexabromocyclododecane (HBCD). |
| 2007 | Editorial Board member of Environmental Toxicology and Chemistry |
| 2007 | Invited Session Chair for Aquatic Toxicity Workshop, Halifax NS. |
| 2006-09 | Member Science Advisory Panel for the metal mining and pulp and paper mills Environmental Effect Monitoring program |
| 2006 | Invited Session Chair for ANCAP / SETAC Africa International Conference on Pesticide Use in Developing Countries, Tanzania |
| 2001 | Invited Session Chair for Annual Society of Environmental Toxicology and Chemistry Meeting, Baltimore, MD |
| 2001 | Invited Session Chair for Aquatic Toxicology Workshop, Winnipeg, MB |
| 1999 | Arnold Naimark Young Investigator Award for excellence in postdoctoral research by a Canadian in the field of Cardiovascular Research |

## Most Significant Contributions

I am an aquatic toxicologist with 25 years of experience in determining exposure, evaluating potential impacts and developing mitigation strategies related to chemical and non-chemical aquatic stressors. I have directed projects examining high-volume and trace industrial chemicals, mining effluents, wastewater components, nutrients and abiotic stressors in aquatic and terrestrial systems. I have also directed multidisciplinary study teams to provide solutions related to mobility, exposure and effects of metals, metalloids (Arsenic, Selenium), brominated and organophosphorous flame retardants, endocrine disruptors, pesticides, PCBs and PAHs. Additionally, I have extensive project experience with multi-level examinations of wastewaters from precious and base metal mines, coal and uranium mines, pulp and paper mills, municipal effluents and in cumulative impact assessments. I have amassed considerable experience with Canada's regulatory framework for Environmental Effects Monitoring (EEM), having served as a longstanding member of the Science Advisory Panel for the metal mining and pulp and paper mill programs. I hAve provided expert advice to the committee on alternative study design methodologies and monitoring issues related to selenium. My expert opinion and testimony has been sought by national and international clients including the United Nations Environment Program, Environment Canada, Health Canada, Natural Resources Canada, the USEPA, World Fisheries Trust, the Swiss National Science Foundation, the Norwegian Ministry of the Environment (MOE), BC MOE, ON MOE, Alberta Sustainable Resource Development, and the Society of Environmental Toxicology and Chemistry.

## Key Publications

### Peer-reviewed publications: 102 since 1991

Stoyanovich, S., Yang, Z., Hanson, M.L., Hollebone, B.P., Orihel, D.M., Palace, V.P., *et al.* 2019. Simulating diluted bitumen spills: Environmental weathering and submergence in model freshwater systems. (*Environ. Toxicol. Chem.*, Accepted July 2019).

Bulloch P, Schur S, Muthumuni D, Xia Z, Johnson W, Chu M, Palace V, Su G, Letcher R, Tomy G. 2019. F2-Isoprostanes in Fish Mucus: A New, Non-Invasive Method for Analyzing Oxidative Stress. Chemosphere (Accepted, August 2019).

Eng ML, Karouna-Renier NK, Henry PFP, Letcher RJ, Schultz SL, Bean TG, Peters LE, Palace VP, Williams TD, Elliott JE, Fernie KJ. 2019. In ovo exposure to brominated flame retardants Part II: Assessment of effects of TBBPA-BDBPE and BTBPE on hatching success, morphometric and physiological endpoints in American kestrels. Ecotoxicol Environ Saf.179:151-159

Stroski KM, Tomy G, Palace VP. 2019. The current state of knowledge for toxicity of corexit EC9500A dispersant: a review. Crit. Rev. Environ. Sci. Tech. DOI: 10.1080/10643389.2018.1532256

Guiguenoa, MF, NK. Karouna-Renier, FP Paula, JA Head, LE Peters, VP Palace, RJ Letcher, KJ Fernie. 2018. Female hatchling American kestrels have a larger hippocampus than males: A link with sexual size dimorphism? Behavioural Brain Research. 349:98-101

McDougall, M.; Francisco, O.; Harder-Viddal, C.; Roshko, R.; Heide, F.; Sidhu, S.; Khajehpour, M.; Leslie, J.; Palace, V.P.; Tomy, G.T.; Stetefeld, J. 2018. Proteinaceous nano container encapsulate polycyclic aromatic hydrocarbons. Scientific Reports. 9:1058.

Anderson JC, Scrimgeor G, Palace VP, Suitor M, Wilcockson J. 2017. Quantifying elements in Arctic Grayling and Bull Trout in the South Nahanni River watershed, Northwest Territories, using nonlethal tissue samples. North Amer. J. Fish. Manag. 37:50-63.

Fernie KJ, Cruz-Martinez L, Peters L, Palace V, Smits JEG. 2017. Inhaling benzene, toluene, nitrogen dioxide, and sulfur dioxide disrupts thyroid function in captive American Kestrels. Environ. Sci. Technol. (In Press: DOI 10.1021/acs.est.6b03026)

Marteinson SC, Palace VP, Letcher RJ, Fernie KJ. 2017. Disruption of thyroxine and sex hormones by 1,2-dibromo-4-(1,2 dibromoethyl)cyclohexane (DBE-DBCH) in American kestrels (*Falco sparverius*) and associations with reproductive and behavioral changes. Environ. Res. (Revised Version under Editorial consideration, Last Undated Dec. 24, 2016)

Anderson JC, Park BJ, Palace VP. 2016. Microplastics in aquatic environments: implications for Canadian ecosystems. Environ. Pollut. 218: 269-280

Bartlett AJ, Struger J, Grapentine LC, Palace VP. 2015 Examining impacts of current-use pesticides in Southern Ontario using in situ exposures of the amphipod *Hyalella azteca*. *Environ. Toxicol. Chem. (Accepted, Sept. 29)*.

Brandt C, Burnett DC, Arcinas L, Palace V, Anderson WG. 2015. Effects of chlorpyrifos on in vitro sex steroid production and thyroid follicular development in adult and larval Lake Sturgeon, *Acipenser fulvescens*. *Chemosphere*. 132:179-187.

Fernie K, Palace V, Peters L, Basu N, Letcher R, Karouna-Renier N, Schultz S, Lazarus R, Rattner B. *2015.* Investigating Endocrine and Physiological Parameters of Captive American kestrels Exposed by Diet to Selected Organophosphate Flame Retardants. *Environ. Sci. Technol.* 49: 7448-7455, 2015.

Arnold MC, Friedrich LA, Ross M, Halden NM, Bernhardt E, Palace VP, DiGiulio RT. 2015. Microchemical Analysis of Selenium in Otoliths of Two West Virginia Fishes Captured near Mountaintop Coal Removal Mining Operations. Environ. Toxicol. 34:1039-1044.

Blanchfield PJ, Kidd KA, Docker M, Palace VP, Park BJ, Postma LD. 2015. Recovery of a Wild Fish Population from Whole-lake Additions of a Potent Estrogen Mimic. Environ. Sci. Technol. 49:3136-3144.

Tomy GT, Halldorson T, Chernomas G, Bestvater L, Dangerfield K, Ward T, Pleskach K, Stern G, Atchison S, Majewski A, Resit JD, Palace VP. 2014. Polycyclic Aromatic Hydrocarbon Metabolites in Arctic Cod (*Boreogadus saida*) from the Beaufort Sea and Associative Fish Health Effects. *Environ. Sci. Technol. 48:11629-11636*.

Anderson JC, Dubetz C, Palace VP. 2014. Neonicotinoids in the Canadian Aquatic Environment: A Literature Review on Current Use Products with a Focus on Fate, Exposure and Biological Effects. *Sci. Tot. Environ. 505:409-422*.

Elliott KH, Welcker J, Gaston AJ, Hatch SA, Palace V, Hare JF, Speakman JR, Anderson, WG. 2013. Thyroid Hormones Correlate with Resting Metabolic Rate, Not Daily Energy Expenditure, in Two Charadriiform Seabirds. *Biology Open. 22:580-586*.

Desforges JP, Rossm PS, Dangerfield N, Palace VP, Whiticar M, Loseto LL. 2013. Vitamin A and E Profiles as Biomarkers of PCB Exposure in Beluga Whales (*Delphinapterus leucas*) from the Western Canadian Arctic. *Aquat. Toxicol. 29:317-328*.

# Curriculum Vitae

**Name/Affiliation:** Mark R. SERVOS, University of Waterloo

## Current Job Title

Professor, Department of Biology, University of Waterloo, 2003-Present

## Key Examples of Awards and Distinctions

- Canada Research Chair (Tier 1) in Water Quality Protection (2004-present)
- Stephen J. Klaine Environmental Education Award, Society of Environmental Toxicology and Chemistry (SETAC; 2019)
- Best Paper Award, Environmental Science and Technology Letters (2019)
- Appointed Fellow of the Society of Environmental Toxicology and Chemistry (2018)
- Synthesis Fellow, Swiss Federal Institute of Aquatic Science and Technology (2018-19)
- Science Council, Water Research Centre for Agriculture and Mining (CRHIAM), Chile (2014-2019)
- Appointed to Grand River Water Plan Science Advisory Committee (2013-14)
- Scientific Director of the Canadian Water Network, NCE in water innovation (2003-11)
- Presidential Citation from SETAC (2002 and 2006) recognizing continued exceptional service
- Ontario Minister of the Environment Advisory Committee on Drinking–Water Quality Stds (2003-04)
- Government of Canada 5-NR Award to Leaders in Sustainable Development (2002)
- President of the Society of Environmental Toxicology and Chemistry (2000-01)
- Environment Canada “Citation of Excellence” (1998)
- President of the International Association of Great Lakes Research (1995-96)
- Canada 125 Medal (1992)

## Research Funding

Since 2012 Dr. Servos has been awarded as PI or co-PI on grants totalling more than $17.2M ($>6.5M directly to Servos). This has included funding from a diversity of sources including NSERC, CFI, federal, provincial and municipal government departments and agencies, Canadian Water Network, GWF and others. *See relationship to other research below.*

## Most Significant Contributions

Prof. Servos is recognized internationally as a leading expert on ecotoxicology and risk of priority and emerging contaminants in the environment. He has published extensively on environmental assessment and biomonitoring of multiple stressors associated with a variety of human activities and provided advice to numerous agencies nationally and internationally.

***1. Assessing the risk of emerging contaminants.*** Servos and colleagues were among the first to document the exposure, effects and risk of emerging contaminants (i.e. estrogens, pharmaceuticals) in the Canadian environment (e.g. 32). He has examined and modelled the fate of these chemicals in wastewater, surface waters and drinking water in watersheds (e.g. 17,31). The co-authored paper on estrogens in wastewater (33) remains a very highly ISI cited paper. This body of work contributed to the formation of national policy and harmonization of risk assessment approaches internationally (e.g. 30). Recent work has explored how emerging contaminants and wastewater cumulatively impact watersheds. Fish exposed to municipal effluents have shown altered gene expression (e.g.,18,29) and production of hormones (6), high levels of intersex (19) reduced reproductive performance (19), as well as changes in fish community assemblages (13,22). Upgrades at wastewater treatment plants have led to a rapid

recovery in several endpoints, including intersex and hormones (6,8,11). This work is now tracking changes and predicting effects of remedial actions (5,7) across levels of biological organization (12) to support national decision makers in biomonitoring and wastewater policy development (e.g. 4).

***2. Impact of industrial effluents and contaminants.*** The work on pulp mill effluent impacts on fish populations was recognized among the most impactful series of studies published in ET&C (25). Process changes in pulp mills resulted in a dramatic decline in organochlorine (e.g. dioxins) and reproductive responses in fish (e.g. 21). Research on the impacts of pulp and paper mills supported the implementation and assessment of new national regulations in this sector as well as development of the EEM program. Work has explored the distribution of organochlorines (e.g. PCBs, dioxins) in fish in the Great Lakes and factors that modified bioavailability of these chemicals in food webs. He has continued to examine how the structure of food webs controls bioaccumulation and biological responses of aquatic organisms in watersheds, large lakes, estuaries and Antarctica.

***3.. Application of Solid Phase Microextraction (SPME) to in vivo sampling in fish.*** Novel extraction techniques using SPME fibres were developed to quantitatively measure contaminants in living fish (in vivo SPME) with minimal effect on their health and applied this novel technology to detect pharmaceuticals, pesticides and novel compounds in fish (e.g. 26). The technique has potential for application in environmental assessments requiring minimal impact on the biota (e.g. threatened species, regulatory testing), and provides a tool for spatial and temporal *in vivo* sampling of animals and environmental matrices (e.g. 10,15). A recent paper on SPME *in vivo* applications for metabolomics won a best paper award from ES&T Letters in 2019 (3).

***4. Novel properties and application of nanoparticles.*** Research has led to the development of novel membranes using titanium dioxide nanowires that are photocatalysts able to remove trace levels of algal toxins, pharmaceuticals and endocrine disruptors (e.g. 1,2,16,23).He has examined how surface properties of silver and gold nanoparticles can be modified to allow rapid synthesis and functionalization with DNA (e.g. 20,27). This work creates opportunities to easily functionalize nanoparticles with DNA sequences that allow them to have diverse applications, including water treatment, biosensing and drug delivery. The paper on citrate-capped gold nanoparticles (24) was selected by the editors as one of the most important articles published in the 30 year history of the Journal Langmuir. This is a very active area of research and development, and these chemicals have implications for the environment as well.

## Relationship to Other Grants

Selected Current Funding:

1) Water Quality Protection (2018 Renewal). Funding Source: Canada Research Chairs. PI: M. Servos, Amount: $1,400,000. Relationship to Current Proposal: The CRC will indirectly support the proposal through access to technical support and infrastructure.
2) Assessing the impact of wastewater treatment plant upgrades on fish health. PI: M. Servos and G. Van Der Kraak. Funding Source: NSERC Collaborative Research and Development Grant (Region of Waterloo). Amount: $321,888. Relationship: Provides sites where the results can be compared.
3) Degradation of emerging water contaminants using novel photocatalytic 3D-printed TiO2 nano-architectures: a case for algal toxins found in harmful algal blooms. Funding Source: NSERC Strategic Grant 2016-2019. PI: N. Zhou and M. Servos, Amount: $580,600. Relationship: None
4) Multiple stressors in watersheds. Funding Source: NSERC Discovery, 2017-2022, PI: M. Servos, Amount: $200,000. Relationship: Foundational bioassessment work directly related to the proposal.
5) Enhanced assessment of multiple stressors. Funding Source: Canada Foundation for Innovation, 2018-2020. PI: M. Servos. Amount: $394,000. Relationship: Infrastructure related to analysis.

6) Linking multiple stressors to adverse ecological responses across watersheds. Funding Source: Global Water Futures (Pillar 1), Canada First Research Excellence Fund (2018-2020). PI: M. Servos, W. Parker, P. Craig. Amount: $300,000. Relationship: Modeling development for bioassessment.
7) Enhancing adaptive capacity and resilience of lakes and their watersheds (Lake Futures). Funding Source: Global Water Futures (Pillar 3), Canada First Research Excellence Fund. PI: Basu et al. Amount: $1,578,252. Relationship: Using some same sites/fish in Grand River.
8) Next generation solutions to ensure healthy water resources for future generations. Funding Source: Global Water Futures (Pillar 3), Canada First Research Excellence Fund. PI: Giesy et al. Amount: $1,391,228. Relationship: Continuation of research theme on environmental DNA.
9) Liposome-encapsulated gold nanocluster for image-guided radiation therapy. Funding Source: Grand River Hospital (IGRT). PI: M. Servos and X. Zhang. Amount: $49,950. Relationship: None.

## Key Publications

Prof. Servos has published over 200 peer reviewed journal articles and book chapters, plus 67 technical publications that have received more than 12,000 citations (currently >1000/y). His Hirsch (H) index is 52 and his *i*10 Index is 142 (Google Scholar®). Selected examples of key publications are listed below.

1. Schneider, O.M., R. Liang, L. Bragg, I. Jaciw-Zurakowsky, … M.R. Servos, Y.N. Zhou. 2019. Photocatalytic degradation of microcystins by TiO2 using UV-LED pulse width modulation. *Catalysts* 9(2): 181.
2. Liang, R., Van Leuwen, J.C., L.M. Bragg, M.J. Arlos, …. M.R. Servos, Y.N. Zhou. 2019. Utilizing UV-LED pulse width modulation on $TiO_2$ advanced oxidation processes to enhance the decomposition efficiency of pharmaceutical micropollutants. Chem. Eng. J. 361:439-449.
3. Roszkowska, A. M. Yu, V. Bessonneau, L. Bragg, M. Servos, J. Pawliszyn. Metabolome Profiling of fish muscle tissue exposed to Benzo[a]pyrene using in vivo SPME. *Environ. Sci. Technol. Letters* 2018 5(7):431-435. Best Paper Award: https://dx.doi.org/10.1021/acs.estlett.9b00161
4. Mavinic, D., S. Arora, C. Brooks, Y. Comeau, M. Darbyshire, K. Kidd, T. McClenaghan, M. Servos. 2018. Canada's Challenges and Opportunities to Address Contaminants in Wastewater. National Expert Advisory Panel Report, Canadian Water Network, Waterloo, Ontario.
5. Arlos, M.J., W.J. Parker, J.R Bicudo, P. Law; K.A. Hicks, M. Fuzzen, S. Andrews, M.R. Servos. 2018. Modeling the exposure of wild fish to endocrine active chemicals: potential linkages of total estrogenicity to field-observed intersex. *Water Research.* 139:187-197.
6. Marjan, P., G.J. Van Der Kraak, D.L. MacLatchy, M.L.M. Fuzzen, L.M. Bragg, M.E. McMaster, G.R. Tetreault and M.R. Servos. 2018. Assessing recovery of in vitro steroid production in male rainbow darter in response to municipal wastewater infrastructure changes. *Environ. Toxicol. Chem*. 37(2):501-514.
7. Arlos, M.J., W.J. Parker, P. Law, J. Bicudo, P. Marjan, S.A. Andrews, M.R. Servos. 2018. Multi-year prediction of estrogenicity in municipal wastewater effluents. *Sci. Total Environ.* 610-611C:1103-1112.
8. Hicks K.A., E.L. McCann, M.K.M. Fuzzen, L. Bragg, S. Kleywegt, G.R. Tetreault, M.E. McMaster, M.R. Servos. 2017b. Rapid reduction of intersex in a wild fish population in response to major municipal wastewater treatment plant upgrades. *Environ. Sci. Technol.* 51(3):1811–1819.
9. Marjan, M.L., M. Fuzzen, D.L. MacLatchy, M.E. McMaster, C.J. Martyniuk, M.R. Servos. 2017b. Returning to normal? Assessing transcriptome recovery over time in male rainbow darter liver in response to wastewater treatment plant upgrades. *Environ.l Toxicol. Chem.* 36(8):2108–2122.
10. Bessonneau V., J. Ings, M. McMaster, R. Smith, L. Bragg, M. Servos, J. Pawliszyn. 2017. In vivo microsampling to capture the elusive exposome. *Scientific Reports* 7: 44038.
11. Hicks, K.A., H.A. Loomer, M.L.M. Fuzzen, S. Kleywegt, G.R. Tetreault, M.E. McMaster, M.R. Servos MR. 2017. $\delta^{15}N$ tracks changes in the assimilation of sewage-derived nutrients into a riverine food web before and after major process alterations at two wastewater plants *Ecological Indicators* 72: 747–758.
12. Fuzzen, M.L.M., L. Bragg, G.R. Tetreault, P.A. Bahamonde, R.N. Tanna, C.J. Bennett, M.E. McMaster, M.R. Servos 2016. An assessment of the spatial and temporal variability of biological responses of rainbow darter collected through an urban gradient. *PloS One* 11 (10), e0164879.

13. Robinson, C.S., G. Tetreault, M.E McMaster, M.R. Servos, 2016. Differential response of two darters species to tertiary treated municipal wastewater effluent in a small receiving environment. *Ecol. Indicators* 60:594-602.
14. Arlos, M.J., M.H. Hatat-Fraile, R. Liang, N. Zhou, S., Andrews, M.R. Servos. 2016. Efficiency of UV-LED activated $TiO_2$ photocatalytic membranes in the treatment of trace organic contaminants including pharmaceuticals and their metabolites. *Water Research* 101:351–361.
15. Bessonneau V., J. Ings, M. McMaster, R. Smith, L. Bragg, M. Servos, J. Pawliszyn. 2016. In vivo tissue sampling using solid-phase microextraction for non-lethal exposome-wide association study of CYP1A1 induction in *Catostomus commersonii*, *Environ. Res.* 151:216–223.
16. Arlos, M.J., R. Liang, M.M. Hatat-Fraile, L.M. Bragg, N.Y. Zhou, M.R. Servos, S.A. Andrews. 2016. Photocatalytic decomposition of selected estrogens and their estrogenic activity by UV-LED irradiated $TiO_2$ immobilized on porous titanium sheets via thermal-chemical oxidation. *J. Hazardous Materials* 318:541-550.
17. Arlos, M.J., L.M. Bragg, W.J. Parker, M.R. Servos. 2015. Distribution of selected antiandrogens and pharmaceuticals in a highly impacted watershed. *Water Research* 72:40-50.
18. Bahamonde, P.A., M. Fuzzen, C.J. Bennett, G.R. Tetreault, M.E. McMaster, M.R. Servos, C.J. Martyniuk, K.R. Munkittrick. 2015. Whole organism responses and intersex severity in rainbow darter following exposures to municipal wastewater in the Grand River basin. *Aquat. Toxico1*.59:290-301.
19. Fuzzen, M.L., C.J. Bennett, G.R. Tetreault, M.E. McMaster, M.R. Servos. 2015. Severe intersex is predictive of poor fertilization success in populations of rainbow darter from the Grand River. *Aquat. Toxicol.* 59:290-301
20. Curry, D., H. Scheller, M. Lu, M. Mkandawire, M.R. Servos, S. Cui, X. Zhang, K.D. Oakes. 2015. Prevention of doxorubicin sorptive losses in drug delivery studies using polyethylene glycol *RSC (Royal Society of Chemistry) Advances* 5(33): 25693-25698
21. Dahmer, S.C., R.I. Hall, K.R. Munkittrick, M.E. McMaster, M.R. Servos. 2014. Historical trends of polychlorinated dibenzo-p-dioxins and dibenzofurans in white sucker liver and dated sediment cores from Jackfish Bay, Lake Superior. *Environ. Toxicol. Chem*. 34 (110) 2489-2502.
22. Tetreault, G.R., C.M. Brown, C.J. Bennett, K.D. Oakes, M.E. McMaster, M.R. Servos. 2013. Fish community responses to multiple municipal wastewater inputs in a watershed. *Int. Environ. Assess. Manag.* 9:456–468.
23. Hu, A., X. Zhang, S. Kurdi, D. Luong, R. Liang, H. Huanga, P. Peng, K. D. Oakes, Y. Zhou, M.R. Servos. 2013. Enhanced photocatalytic degradation of dyes by TiO2 nanobelts with hierarchical structures. *J. Photochem. Photobiol. A* 256:7-15.
24. Zhang, X, M.R. Servos, J. Liu. 2012. Surface science of DNA adsorption onto citrate-capped gold nanoparticles. *Langmuir* 28:3896-3902. *Recognized one of the most impactful papers in Langmuir*
25. Munkittrick, K.R., M. McMaster, M.R. Servos. 2012. Detection of reproductive impacts of effluents from pulp and paper mills. *Environ. Toxicol. Chem*. 32:729–731. *Recognition of impact in ETC.*
26. Zhang, X., K.D. Oakes, S. Wang, S. Cui, J. Pawliszyn, M.R. Servos. 2012. In vivo sampling of environmental organic contaminants in fish by solid-phase microextraction. *Trends Anal.Chem.*31:32-39.
27. Zhang, X, B. Liu, N. Dave, M.R. Servos, J. Liu. 2012. Instantaneous attachment of an ultrahigh density of non-thiolated DNA to gold nanoparticles and its applications. *Langmuir 2*8:17053-17060.
28. Tetreault, G., C. Bennett, K. Shires, B. Knight, M. Servos, M. McMaster. 2011. Intersex and reproductive impairment of two species wild fish exposed to multiple municipal wastewater discharges in Canada. *Aquatic Toxicology* 104:278-290.
29. Ings, J., M. Servos, M. Vijayan. 2011. Hepatic transcriptomics and protein responses to in situ municipal wastewater effluent exposure in rainbow trout. *Environ. Sci. Technol.* 45:2368-2376
30. Oakes, et al. 2010. An environmental risk assessment for the serotonin re-uptake inhibitor fluoxetine - a case study utilizing the European risk assessment framework. *Int. Environ. Assess. Man.* 6:524-539.
31. Rahman, M.F., E.K. Yanful, S. Y. Jasim, L. Bragg, M.R. Servos, S. Ndiongue, D. Borikar. 2010. Advanced oxidation treatment of drinking water. *Ozone Sci. Eng.* 32:217-229. *Harvey Rosen Award.*
32. Servos M.R., D.T. Bennie, B.K. Burnison, A. Jurkovic, R. McInnis, T. Neheli, A. Schnell, P. Seto, S.A. Smyth, T.A. Ternes. 2005. Removal of hormones, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants. *Sci. Total Environ.* 336:155-170.
33. Ternes, Th.A., M. Stumpf, J. Mueller, K. Haberer, R.-D. Wilken and M. Servos. 1999. Behavior and occurrence of estrogens in municipal sewage treatment plants. *Sci. Total Environ.* 225:81-90.

# Xiaowei Zhang, PhD

Professor, Director of Research Centre
School of the Environment, Nanjing University, Nanjing, Jiangsu, China, 210046
Email: zhangxw@nju.edu.cn

**Education:**

PhD, Zoology & Environmental Toxicology (2008) Michigan State University, East Lansing, USA.
MSc, Applied Statistics (2007), Michigan State University, East Lansing, USA.
PhM, Environmental Science (2003), City University of Hong Kong, Hong Kong, P.R. China.
BSc (Hons), Environmental Biology (2000), Nanjing University, P.R. China.

**Professional Appointments:**

***Professor***, School of the Environment, Nanjing University, Nanjing, Jiangsu, China, 2010-present
***Research Associate***, Toxicology Centre, Univ. Saskatchewan, Saskatoon, SK, Canada, 2009-2010

**Recent Awards**

- ACS ES&T Early Career Scientist (2019)
- The 1st Young Scientist Award of Chinese Society for Environment Sciences (2018)
- Youth Science and technology innovation leader, Ministry of Science and Technology (2018)
- Youth Changjiang Scholar (2016), the highest academic award issued to an individual who is less than 38 years old by the Ministry of Education of the People's Republic of China.

**Evidence of Impact:** 125 peer-reviewed publications. > 2500 total citation, H-Index 31.

**Service and Outreach:**

Associate Editor-in Chief—Asian Journal of Ecotoxicology; Editor—Environmental Toxicology and Chemistry, Environment Science Europe, Bulletin of Environment Chemistry and Toxicology; Associate Editor—Chemosphere; Editorial Board-- Science of Total Environment; Member of SETAC AOP advisory committee, Member of SETAC OMICS advisory committee; Director—Centre of Chemical Toxicology and Environmental Safety, Nanjing University; Associate Director—National Engineering Centre on Pollution Control and Resource Reuse, Nanjing University;

**Selected Research Funding:**

**Xiaowei Zhang** (PI, 2017-2020) Predictive Toxicology and Mechanistic Study on Developmental Toxicity of Two Groups of Persistent AhR Active Chemicals in Zebrafish, National Natural Science Foundation of China (NSFC). (¥670,000) $100,000.

**Xiaowei Zhang** (PI, 2013-2016) "Integrated Application of Chemical risk identification and control in Chemical Industry Zone along the Yangtze River, China" National High-tech R&D Program of China (863 Program) (¥10,800,000) $1,753,000.

**Xiaowei Zhang** (PI, 2013-2016). Development of Chemical Toxicogenomics. Award Grant for Excellent Young Scientist from National Natural Science Foundation of China (NSFC) (¥1,000,000) $163,000.

**Xiaowei Zhang** (Co-PI, 2013-2018). Toxico-genomic Assessment of Emerging Environmental Pollutants Using Novel Functional Genomic and High Throughput Technologies, sub project of Solutions for present and future emerging pollutants in land and water resources management. European Commission, (€350,000) $453,270.

**Xiaowei Zhang** (PI, 2014-2017). Development of novel tools to assess and predict the ecological risks posed by chemical pollution by integration of DNA barcode based technology. Award grant for Distinguished Young Scholars, National Science Foundation of Jiangsu Province. (¥1,000,000) $163,000.

**Selected Publications:**


1. **Zhang X***. Environmental DNA Shaping a New Era of Ecotoxicological Research. 2019. Environ Sci Technol. 53(10):5605-5612. (**Invited ES&T Perspective**)
2. **Zhang X*,** Xia P, Wang P, Yang J, Baird D. 2018. Omics advances in Ecotoxicology. *Environ Sci Technol*. 52(7):3842-3851. (**Invited ES&T Feature Article***)*
3. Fang W, Peng Y, Muir D, Lin J, **Zhang X***. 2019. A critical review of synthetic chemicals in surface waters of the US, the EU and China. Environ Int. 131:104994.
4. Corcoll N*, Yang J, Backhaus T, **Zhang X*,** Eriksson KM, 2019. Copper affects composition and functioning of microbial communities in marine biofilms at environmentally relevant concentrations. Front Microbiol. 9, 3248.
5. Yang J, Jeppe KJ, Pettigrove VJ, **Zhang X***. 2018. eDNA metabarcoding supporting community assessment of environmental stressor in a field-based sediment microcosm study. Environ Sci Technol. 52(24):14469-14479.
6. Li F, Peng Y, Fang W, Altermatt F, Xie Y, Yang J, **Zhang X*.** 2018. Application of environmental DNA metabarcoding for predicting anthropogenic pollution in rivers. *Environ Sci Technol*. *52* (20):11708–11719.
7. Xie Y, **Zhang X*,** Yang J, Kim S, Hong S, Giesy JP, Yim UH, Shim WJ, Yu H, Khim JS*. 2018. eDNA-based bioassessment of coastal sediments impacted by an oil spill. Environ Pollut. 238:739-748.
8. Xie Y, Floehr T, **Zhang X*,** Xiao H, Yang J, Xia P, Burton GA Jr, Hollert H. 2018. In situ microbiota distinguished primary anthropogenic stressor in freshwater sediments. Environ Pollut. 239:189–197
9. Wang P, Xia P, Yang J, Wang Z, Peng Y, Shi W, Villeneuve DL, Yu H, **Zhang X***. 2018. A Reduced Transcriptome Approach to Assess Environmental Toxicants Using Zebrafish Embryo Test. *Environ Sci Technol*. 52(2):821-830.
10. Yang J, **Zhang X***, Zhang W, Sun J, Xie Y, Zhang Y, Burton GA Jr, Yu H. 2017. Indigenous species barcode database improves the identification of zooplankton. PLoS One. 12(10): e0185697.
11. Yang J, Xie Y, Jeppe K, Long S, Pettigrove V, **Zhang X***. 2018. Sensitive community responses of microbiota to copper in sediment toxicity test. Environ Toxicol Chem. 37(2):599-608.
12. Guan M, Fang W, Ullah S, **Zhang X***, Saquib Q, Al-Khedhairy AA. 2018. Functional genomics assessment of narcotic and specific acting chemical pollutants using E. coli. Environ Pollut. 232:146-153.
13. Guan M, **Zhang X***, 2017. Functional genomic assessment of 2, 2-bis (bromomethyl)-1, 3-propanediol induced cytotoxicity in a single-gene knockout library of of *E. coli*. Chemosphere, 185: 582-588.
14. Morandi GD, Wiseman SB, Guan M, **Zhang XW***, Martin JW, Giesy JP*. 2017. Elucidating mechanisms of toxic action of dissolved organic chemicals in oil sands process-affected water (OSPW). Chemosphere. 86:893-900.
15. Xia P, **Zhang X***, Zhang H, Wang P, Tian M, Yu H. 2017. Benchmarking Water Quality from Wastewater to Drinking Waters Using Reduced Transcriptome of Human Cells. *Environ Sci Technol*. doi: 10.1021/acs.est.7b02648.
16. Yang J, **Zhang X***, Xie Y, Song C, Sun J, Zhang Y, Giesy JP, Yu H. 2017. Ecogenomics of Zooplankton Reveals Ecological Threshold of Ammonia Nitrogen. *Environ Sci & Technol. 51: 3057–3064*.
17. Yang J, **Zhang X***, Xie Y, Song C, Yu H, Burton GA. 2017. Zooplankton Community Profiling in a Eutrophic Freshwater Ecosystem-Lake Tai Basin by DNA Metabarcoding. *Sci Rep.* 7: 1773

18. Shi W, Yu N, Jiang X, Han Z, Wang S, **Zhang X***, Wei S, Giesy JP, Yu H*. 2017. Influence of blooms of phytoplankton on concentrations of hydrophobic organic chemicals in sediments and snails in a hyper-eutrophic, freshwater lake. *Water Res.* 113:22-31.
19. Peng Y, Xia P, Zhang J, Villeneuve DL, Zhang J, Wang Z, Wei S, Yu H, **Zhang X***. 2016. Toxicogenomic Assessment of 6-OH-BDE47 Induced Developmental Toxicity in Chicken Embryo. *Environ Sci Technol*. 50(22):12493-12503.
20. Xia P, **Zhang X***, Xie Y, Guan M, Villeneuve DL, Yu H. 2016. Functional Toxicogenomic Assessment of Triclosan in Human HepG2 Cells Using Genome-Wide CRISPR-Cas9 Screening. *Environ Sci Technol*. 50(19):10682-10692.

**Significant contributions to research:**

*Continued Development of -Omics Assays for Chemical Hazard Assessment.* Throughout my research career I have addressed an ongoing need for the development of novel techniques to reduce cost, time and animal use related to chemical toxicity assessment. With funding from the US EPA, my PhD research had generated the H295R *in vitro* method to screen for the potential of chemicals to affect the steroidogenesis of endocrine system (Hilscherova et al. 2004; Zhang et al. 2005, US patent #08299238). These techniques have been adopted by OECD and the US EPA to test and screen chemicals. Another high throughput assay I have developed for chemicals and mixture testing is a microbial genome wide live cell reporter array system (Zhang et al., 2011). Being a rapid, accurate and cost-effective method of chemical test, this novel system offers scope for a nontargeted perspective to identify potential mechanism of chemical toxicity. This assay was selected as one of the novel functional genomic and high throughput technologies, used in to provide "Solutions for present and future emerging pollutants in land and water resources management" (European Commission FP7 project). The latest development in my group was the functional genomic CRISPR-Cas9 screening for chemical safety assessment (Xia et al., 2016). A new functional genomics screening technology which employed CRISPR-Cas9 was developed to characterize the interaction of chemical and human genes, which provides a new alternative assay to assess chemical toxicity. This work was published in October 2016 as the cover of ES&T, which was also selected as the American Chemical Society (ACS) Editor 's Choice with free open assess granted.

*Development of AOP model to predict toxicology of emerging environmental chemicals.* Through the genome-wide live cell array screen technology, our works revealed that the hydroxylation might be the toxicification mechanism of polybrominated diphenyl ethers (PBDEs) and their analogues (Su et al., 2012). We were the first group reported that the OH-BDE and PCDPSs activate aromatic hydrocarbon receptor (AHR) mediated molecular mechanisms and thereby might cause potential developmental toxicity. A chemical predictive toxicology model based on AHR AOP was established. Then an integrated test system consisting of QSAR, high-throughput screening, cell transcriptomics technology, high content screening and phylogenetic analysis were used in testing, screening and identification of toxic chemicals. We found that PCDPSs can activate the activity of aryl receptors and induce oxidative stress pathway in living cells which has the potential of embryonic development toxicity and potential ecological hazard. At the same time, the embryo developmental toxicity of 6-OH-BDE47 was predicted and verified (Peng et al., 2016). These results provide theoretical basis and technical support for toxicity identification and risk prediction of emerging pollutants.

*Development of the next generation of biodiversity and functional assessment of aquatic ecosystem by Ecogenomics.* I initiated the first project in China to develop the next generation ecogenomics technologies to provide high throughput monitoring and assessment of biodiversity in rivers and lakes. In this project, a set of advanced environmental DNA meta-barcoding methods (eDNA) were developed to provide powerful and unbiased tools for fine-scale monitoring of biodiversity in ecosystems. These new technologies have already been adopted by the Jiangsu Provincial program of monitoring aquatic systems, which has started to

revolutionize or improve the environment management practice for aquatic ecosystem, such as river and lake in China and the rest of world.

**Letters of Support: Giesy et al**

1. Agriculture and Food Canada (federal)
2. Six Nations of the Grand River
3. Alberta Irrigation Districts Association (Alberta Ag and Forestry)
4. Alberta Environment and Parks (Fred Wrona, Chief Scientist)
5. Saskatchewan Ministry of the Environment, Fish and Wildlife Branch
6. Ontario Ministry of Environmental Conservation and Parks
7. Ontario Ministry of Natural Resources and Forestry
8. Grand River Conservation Authority
9. Experimental Lakes Area (Vince Palace)
10. Orano Resources Canada
11. Trout Unlimited of Canada
12. Middle Grand Chapter of Trout Unlimited
13. Friends of Grand River
14. Ontario Federation of Anglers and Hunters
15. Rare Charitable Reserve
16. Southern Ontario Water Consortium
17. Tai Lake Riverine Pollution Prevention Management Office
18. Nanjing University (Prof Xiaowei Zhang)
19. RECETOX, Masaryk University, Czech Republic, Klara Hilscherova
20. Melimoyu Ecosystem Research Institute

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Science and Technology Branch | Direction générale des sciences et de la technologie

Dr. John P. Giesy
Dept. Veterinary Biomedical Sciences
Toxicology Program Faculty, Toxicology Centre
University of Saskatchewan
Email: john.giesy@usask.ca

September 4, 2019

Re: Renewal of *Next Generation Solutions to Ensure Healthy Water Resources for Future Generations* proposal under Global Water Futures Project

Dear Dr. Giesy,

This letter is to confirm support for the renewal of your project entitled *Next Generation Solutions to Ensure Healthy Water Resources for Future Generations.* Agriculture and Agri-Food Canada (AAFC) has been impressed with the accomplishments of the project so far and look forward to the completion of the work over the next phase.

AAFC works to provide Canadian agriculture with information, research, technology, policies and programs to promote sector competitiveness at home and abroad. As well, the Department helps the sector manage risk and embrace innovation. AAFC's Science and Technology Branch (STB) supports new areas of opportunity for the sector, to supporting sector competitiveness. AAFC is an important provider of science and technology related to water sciences and water management in agriculture in order to improve the quality of life of Canadians.

The goals of this Global Water Futures project complement the interests of AAFC, namely in improving techniques to monitor biodiversity in agricultural ecosystems and the sustainability of the agricultural sector This will directly address strategic goals related to sustainable food supplies and resilient agri-ecosystems, and aligns with AAFC's objectives of helping the sector adjust to climate change, better address water and soil conservation and development issues and make our resource sectors world leaders in the use of clean and sustainable technology and processes.

In the first years of this project, progress has been made towards developing eDNA technologies and standard operating procedures for assessing biodiversity in aquatic ecosystems, particularly those impacted by cumulative effects of agricultural and urban runoff. These techniques form a basis for assessing changes in biodiversity from changing climate and evaluating the efficacy of best management practices designed to improve the sustainability of agricultural ecosystems. Based on recommendations from AAFC and other organizations, this project has expanded its list of partners, including provincial agencies, non-government organizations and industry to work towards improved drinking and agricultural irrigation waters. They have also completed projects on effects of contaminants such as dilbit and selenium on aquatic communities and developed monitoring programs for invasive species and endangered species.

AAFC (through the Agroclimate, Geomatics and Earth Observation Division) has served, and will continue to serve if this renewal is successful, on the Advisory Board to ensure the project remains

aligned and best meets AAFC's strategic objectives. AAFC looks forward to further collaboration opportunities with this project as it develops.

Global Water Futures is the largest university-led water research program ever funded worldwide and is a landmark program aimed at increasing Canada's scientific and technological capabilities to benefit Canadians and communities in other cold regions where climate change is impacting landscapes, ecosystems and the water environment. The project *Next Generation Solutions to Ensure Healthy Water Resources for Future Generations* is a well developed research project that will support scientific advancement in Canada's agriculture and agri-food sector. I encourage GWF to give the project continued support.

Sincerely,

Dr. Javier Gracia-Garza
Director General, Ontario-Quebec Region
Science and Technology Branch
Ottawa, Canada

cc: Dr. Alain Houde, Director- Research, Development and Technology, Saint-Jean-sur-Richelieu, Agri-environment Resiliency at the Ottawa Research and Development Centre

Dr. Andrew Davidson, acting Associate Director Agro-Climate, Geomatics and Earth Observation Division (ACGEO)

François Chretien, Associate Director, Living Labs Initiative

Alberta Agriculture and Forestry

**Primary Agriculture/Irrigation and Farm Water**
J.G. O'Donoghue Building
303, 7000 – 113 Street
Edmonton, Alberta T6H 5T6
Canada
Telephone: 780-644-1971

September 13, 2019

**John P. Giesy**, Ph.D.
Professor & Canada Research Chair in Environmental Toxicology
Department of Veterinary Biomedical Sciences
Toxicology Program Faculty, Toxicology Centre
University of Saskatchewan
44 Campus Drive
Saskatoon, SK, Canada S7N 5B3

Dear Professor Giesy,

We are pleased to provide this letter of support to accompany your joint application for a grant of the Global Water Futures program. The proposal entitled: *Next generation solutions to ensure healthy water resources for future generations*, which is being submitted for renewal to Global Water Futures, is important to advance research on how to manage threats to Canada's water supply and quality. The proposed research project fits closely with the mandate and business outcomes of Alberta Agriculture and Forestry (AAF), and specifically our Water Quality Section.

AAF strives to ensure that Alberta's agriculture and forest industries are innovative, diversified and competitive, as well as ensuring that assurance systems are effective and Alberta's water resources are managed in an environmentally sustainable manner. As part of that mandate, the Water Quality Section (WQS) of AAF works closely with agricultural commodity groups and the irrigation industry to ensure that Alberta's agriculture sector has access to good quality water and uses proven practices to enhance our water resources. The WQS conducts applied water quality research to provide science-supported policy recommendations to the agriculture industry and the Government of Alberta. Since resources are limited, and the demands for information are great, it is of advantage to build mutually beneficial partnerships to meet these needs in a timely and cost-effective manner. Our current partnership with Dr. Giesy under his Global Water Futures grant, is critical to addressing one of our current challenges of how to monitor and interpret the relationships between nutrient availability and plankton communities within the irrigation districts. Algae growth in irrigation conveyance works reduces the efficiency of water delivery and can have serious implications on food production. Of particular concern is the increase of cyanobacteria blooms in irrigation reservoirs. These blooms also introduce public health risks as the irrigation water distribution system provides water for municipal, recreational and livestock watering uses, along with food production. We are very interested in utilizing the eDNA work proposed by Professor Giesy and his colleagues at the University of Saskatchewan and Waterloo to develop management strategies to mitigate the risks proposed by cyanobacteria and other algae. Traditional methods of monitoring cyanobacteria in algae in aquatic environments are resource-intensive and have long turnaround times, as experts highly trained in visual taxonomy must perform the work.

The WQS is excited to be collaborating with Professor Giesy and his team on this project. We are willing to provide expertise and give suggestions on what the most relevant research would be that would deliver the greatest benefit to our programs and our stakeholders. We also are willing to contribute samples collected in association with our existing monitoring programs and data derived from those programs to Prof. Giesy and his team. Professor Giesy has indicated a willingness to share all of the results of his research with our Ministry. We are partnered with the Alberta Irrigation District Association on a multi-year water quality monitoring program that is expected to continue indefinitely. The purpose of the program is to assess the quality of water in Alberta's irrigation districts by measuring ambient concentrations of various chemical and biological parameters and using the data to address potential risks to water quality through management changes. We are working with Prof. Giesy to apply eDNA metabarcoding methods for the purposes of characterizing plankton communities and their relationship with nutrient availability and cyanobacteria growth. This project started in June 2019.

At this time, AAF is unable to offer any direct financial assistance to the research program. However, we do offer in-kind support in the form of field sample collection and providing technical input to make the program as successful as possible. In-kind support, primarily in the form of salaries and field travel expenses, from AAF to the program would be in the range of $75,000 over a three year period. Because we routinely access irrigation sampling sites, the cost of which would be prohibitive to the GWF program, our contribution of samples from such sites would represent significant value and result in substantial financial efficiency for Professor Giesy's renewal proposal.

I, on behalf of the Water Quality Section of AAF, fully support and endorse the research proposed by Professor Giesy under the GWF program. We are willing to collaborate by providing expertise and where appropriate, samples, and in the end, will make use of the information to mitigate the risks that threaten Canada's water supply and quality.

Sincerely,

Greg Piorkowski, Ph.D., P.Ag.
Watershed Research Scientist
Acting Director, Water Quality Section
Alberta Agriculture and Forestry
303, 7000 – 113 Street
Edmonton, Alberta, Canada, T6H 5T6

# Memorandum of Understanding
# regarding analyses of water by Global Water Futures, in partnership with Alberta Irrigation Districts Association

## Date: June 24, 2019

This Memorandum of Understanding is between "Next generation solutions to ensure healthy water resources for future generations" project of Global Water Future program at the University of Saskatchewan (hereafter, eDNA project), Alberta Irrigation Districts Association (AIDA), and Alberta Agriculture and Forestry (AAF).

**Purpose**:
The purpose of this Memorandum of Understanding is to document the collaboration, data ownership and data usage (presentation/publication) arising from projects jointly administered by eDNA project, AIDA, and AAF.

**Background**:
AIDA and AAF are supporting Alberta's irrigation districts in a multi-year water quality program, which is continuing in 2019. The purpose of the program is to assess the quality of water in the irrigation districts by measuring ambient concentrations of various chemical and biological parameters. Specific interest was expressed in partnering on spinoff projects of mutual interest to GWF, AIDA, and AAF.

**Principles:**
Specific project objectives are identified in project summary documents drafted by the project partners before the commencement of projects. The data collected will be used exclusively to resolve the objectives, as outlined in the project summary and agreed upon by all collaborating partners. All project partners will be consulted and acknowledged in public presentations and publication of data. Information not relevant to the interpretation of the data (e.g. specific sample site locations) will be excluded unless agreed upon by all collaborating partners. The use of data outside the scope of individual projects may be undertaken with consultation and agreement by all collaborating partners.

**Scope:**
eDNA project will conduct all laboratory analyses in accordance with appropriate analytical research methods and standardized protocols. Laboratory analysis costs will be covered by eDNA project. AAF and AIDA/irrigation districts will collect and provide all irrigation water samples for analyses and eDNA project will provide all bottles and transportation to the laboratory. AAF will provide a tentative sampling schedule, which details the number of samples and sampling dates. As sampling is weather dependent, AAF will confirm sampling and shipping dates with eDNA project at least two days in advance. AAF will ship samples as per instructions provided by eDNA project.

This memorandum of understanding maybe terminated upon mutual consent of all partners.

Margo Redelback, Executive Director
Alberta Irrigation Districts Association

Acting for

Andrea Kalischuk, Director
Water Quality Section
Alberta Agriculture and Forestry

per J.P.S.

John P. Giesy
eDNA project of Global Water Futures
University of Saskatchewan

Alberta Environment and Parks

**Environmental Monitoring and Science Division**
10 Floor, 9888 Jasper Avenue
Edmonton, AB T5J 5C6
Canada
Telephone 780-229-7270
www.alberta.ca

September 16, 2019

John P. Giesy, Ph.D.
Professor & Canada Research Chair in Environmental Toxicology
Dept. Veterinary Biomedical Sciences
Toxicology Program Faculty, Toxicology Centre
University of Saskatchewan
44 Campus Drive
Saskatoon, Saskatchewan
S7N 5B3

Dear Professor Giesy:

We are pleased to provide this letter of support to accompany the joint application led by Dr. John P. Giesy (University of Saskatchewan) and seven co-investigators from the University of Waterloo and University of Saskatchewan for a Global Water Futures program grant over the period 2020-2023. Their proposal, entitled *Next generation solutions to ensure healthy water resources for future generations*, which is being submitted to the Global Water Futures program being administered by the Global Institute for Water Security at the University of Saskatchewan. The proposed program of research aligns with the mandate and current research interests and direction of Alberta Environment and Parks' Environmental Monitoring and Science Division (AEP; EMSD).

AEP is responsible for protecting the environment of Alberta. As part of that mandate, EMSD is responsible for monitoring for status and trends in a variety of chemical, physical and biological attributes in Alberta's air and aquatic and terrestrial ecosystems. Part of that monitoring includes biotic integrity and biodiversity in aquatic environments. One of our current challenges is how to monitor and interpret changes under a changing climate. An additional stressor to aquatic systems in Alberta is invasive species that can alter environments so that ecological services important to humans and the environment are negatively impacted. Given the demand for monitoring and the costs involved, we are currently looking to meet those needs in a scientifically robust but timely and cost-effective manner and as such, are very interested in seeing the results of the eco-omics work outlined in this research proposal.

One of our primary interests in the proposed eco-omics work is in identifying whether and how more rapid and cost-effective, modern techniques can be applied in our future monitoring programs. However, a number of issues and open questions remain about how to best incorporate new approaches and methods into our monitoring programs without losing scientific integrity or impairing our ability to integrate new information with that produced via older methods. We think the proposed methods are promising and if the research validates the methods we would certainly be interested in assessing their utility in our environmental monitoring and assessment programs. Thus, we could be an enthusiastic end-used of the information.

....2

EMSD is strongly interested in collaborating with the research team on this project. We are willing to provide expertise and give suggestions on what the most relevant research would be that would deliver the greatest benefit to our programs. We also are willing to contribute samples, where appropriate, collected in association with our existing monitoring programs and data derived from those programs. Professor Giesy has indicated a willingness to share all of the results of this research with our ministry.

At this time, EMSD is unable to offer any direct financial assistance to the research program. However, we can offer in-kind support and I estimate that this support from AEP-EMSD to the program would be in the range of $100,000 over a three-year period. Because we routinely access remote sampling sites across the Province, the cost of which would be prohibitive to the GWF program, our contribution of samples from such sites would represent significant value and result in substantial financial efficiency for the proposed research.

I, and EMSD, on behalf of AEP, support and endorse the research proposed under the GWF program. We are willing to collaborate by providing expertise and where appropriate, samples and in the end, will make use of the information that devolves from the program.

Sincerely,

Frederick J. Wrona, Ph.D.
Chief Scientist and Assistant Deputy Minister

Cc: John Orwin, Acting Executive Director
Science Branch, EMSD, AEP

September 1, 2019

John P. Giesy, Ph.D., FRSC, FSETAC
Professor & Canada Research Chair in Environmental Toxicology
U of S Ambassador of Science to China
Dept. Veterinary Biomedical Sciences
Toxicology Program Faculty, Toxicology Centre
University of Saskatchewan
44 Campus Drive
Saskatoon SK S7N 5B3, Canada

Dear Professor Giesy,

We write to offer continued strong support for your research program "Next generation solutions to ensure healthy water resources for future generations". Over the past several years, we have worked closely with your team to develop environmental DNA methods to screen for aquatic invasive species. Using a targeted approach, more than 50 waterbodies were tested and so far, Saskatchewan remains zebra mussel free. The development of eDNA methods has made an important contribution to our Aquatic Invasive Species Strategy by offering a rapid, low cost and sensitive tool to test waters for priority invasive species in the province. Continued vigilance using tools such as eDNA methods will assist in early detection and management.

In the next phase of the research, we hope to continue to assist in refining and applying the eDNA method for invasive species, and we also wish to see an expansion of the toolkit to enable detection of rare and at-risk species in the province. The use of targeted sequencing is a promising approach that will allow multiple species to be screened for at once. Several species of interest such as Chestnut lamprey are particularly elusive with conventional gear types, and mapping the known range of this species will be important for conservation efforts. Other species, including Lake sturgeon, are more widely distributed in the province but we lack the ability to comprehensively map this distribution in relation to particular habitat features that might be worthy of protection. The large volume of samples that can be processed using targeted sequencing will increase our capacity to detect these species and identify critical habitat features.

To contribute to the program, we can offer support in the form of collection of water samples from our fisheries assessment program. Several of our staff have already been trained by your team to follow protocols for collection, filtration and storage of samples. Given the remote nature of some of these locations, we estimate this contribution to be worth $25,000 annually. We can also commit staff time of some of our biologists to assist in developing sampling strategies, reviewing documents, attending

meetings and advising graduate students.  This amounts to a contribution of $10,000 per year. We look forward to continuing this productive collaboration.

Sincerely,

Matt Tyree
Fisheries Unit Manager
Fish, Wildlife and Lands Branch
Saskatchewan Ministry of Environment

**Ministry of the Environment, Conservation and Parks**

Technical Assessment and Standards Development Branch
40 St. Clair Avenue West
7th Floor
Toronto ON M4V 1M2
Phone: 416.327.5519
Fax: 416.327.2936

**Ministère de l'Environnement, de la Protection de la nature et des Parcs**

Direction des évaluations techniques et de l'élaboration des normes
40, avenue St. Clair Ouest
7e étage
Toronto, ON M4V 1M2
Tél: 416 .327.5519
Téléc: 416. 327.2936

Ontario

September 19, 2019

To: Mark Servos
Canada Research Chair in Water Quality Protection
Dept. of Biology, University of Waterloo
200 University Ave W.,
Waterloo Ontario, N3A2L4
mservos@ uwaterloo.ca

RE: Global Water Futures: Next generation solutions to ensure healthy water resources for future generations.

Dear Dr. Servos;

The mandate of the Ontario Ministry of the Environment, Conservation and Parks (MECP) is to protect our air, land and water leading to healthier communities and economic prosperity. In addition, we are committed to using the best available science and research to develop and deliver policies, legislation, regulations, standards, programs and services. The opportunity to support and collaborate with academic research groups in fulfilling these goals is of great importance to our Ministry. Our Environmental Sciences and Standards Division (ESSD) is particularly interested in novel application of emerging technologies supporting environmental monitoring, risk assessment and bioassessment approaches.

We were highly supportive of this project at the conceptual stage and are pleased to see the progression over the last year. The results of the research program proposed by Dr. John P. Giesy (PI), in collaboration with the team at the University of Waterloo (e.g. Dr. Servos et al.), within the Global Water Futures program has the potential to assist the Ministry in fulfilling our mandate as it pertains to the regulation of chemicals and placing them into the context of natural variability and climate change. The techniques being developed in the project may have broad application is assessing changes in distributions of rare, threatened and key managed species in Ontario watersheds, as well as applications in assessing biodiversity.

We are very interested to see this research progress, as it moves from method validation activities and limited field testing in small, single streams, to more field-based projects looking at multiple systems feeding into the Grand River. The usefulness of these approaches in potentially determining impacts to aquatic life from pollution sources such as wastewater treatment plants is of most interest to ESSD. However, other parts of the Ministry will be well served with new tools that help determine species abundance and community structure, especially for species at risk.

Furthermore, the approach by the research group to incorporate traditional knowledge with new innovative technology supports Ontario's commitments under the Canada-Ontario Agreement

on Great Lakes Water Quality and Ecosystem Health. We therefore strongly support the project as we see it as having long term potential to influence and support many of our programs.

As noted in our previous supporting letter, we have collaborated with Dr. Servos and the group at the University of Waterloo in the past on several studies. We see this current research effort as having the potential to link to, and enhance, the studies on impacts of various stressors (e.g. wastewater) that we have been actively collaborating on. We continue to be impressed with the knowledge and professionalism of the team in communicating and presenting their past results to staff here at MECP.

My staff and I look forward to collaborating and working directly with the research team through this very interesting and valuable research project. We will provide both scientific and technical guidance to the project team related to environmental quality standards and risk assessment.

Please feel free to contact me directly if you wish to discuss the Ministry's interest in this research project.

Tim Fletcher
Manager, Water Standards Section
Ministry of the Environment, Conservation and Parks#

**Ministry of Natural Resources and Forestry**

**Ministère des Richesses naturelles et des Forêts**

Guelph District
1 Stone Road West
Guelph, Ontario
N1G 4Y2

Telephone: (519) 826-4955
Facsimile: (519) 826-4929

Ontario

August 27, 2019

Mark R Servos
Department of Biology
University of Waterloo
200 University Avenue West
Waterloo, ON N2L 3G1

Dear Mark Servos,

***RE: Support for proposal, John P. Giesy et al., Next generation solutions to ensure healthy water resources for future generations.***

The Ontario Ministry of Natural Resources and Forestry (OMNRF) is responsible for the sustainable management of fish, wildlife and Crown forest resources in Ontario. In addition, the OMNRF is responsible for the management of Ontario's Crown lands, water, oil, gas, salt, and aggregates resources. The Ministry monitors and protects Ontario's biodiversity through policies, programs, strategies and legislation, in cooperation with a broad range of partners.

We are happy to have participated in the first phase of this research program and strongly endorse the second phase, which has an emphasis on assessing and interpreting eDNA data from streams. During the first two years of the project the research focused on several brook trout streams in Grand River watershed, southern Ontario. This is a unique habitat that results from the upwelling of cold water from the glacial deposits of the Waterloo Moraine. Brook trout are a species of great interest because of their sensitivity to habitat change and possibly climate change. Development of new tools such as Environmental DNA (eDNA) will give us greater ability to assess brook trout populations and determine management actions and policies.

Staff at OMNRF have provided access to historical fisheries data for these streams to help the team select and design the research. We have provided advice and suggestions for enhancing the research outcomes. For the next phase we will continue to contribute in a similar way by providing access to the OMNRF data on fish communities in selected streams to support the research. We will participate in individual and formal meetings (such as the Grand River Recreational Fisheries Implementation Committee) to provide advice. We also review and issue scientific collection permits and have worked with the team to ensure the research is possible in these streams during the required time frames. The work on Washington, Alder, Bauman and Blair Creeks has provided valuable information. It has helped to better define the fate of eDNA and make connections between fish abundance and eDNA collections. In addition, the research team has explored how eDNA is influenced by water quality, and the potential issue it presents for the interpretation of eDNA results.

We support the extension of the research into additional sites, such as Mill Creek and McKenzie Creek, where there is considerable interest in fish populations but little available monitoring data. A better understanding of how to apply and validate eDNA data will be extremely beneficial to all fisheries managers. The planned research supports the Ontario Biodiversity Strategy to address Ontario's biodiversity challenges by improving awareness and understanding of biodiversity.

Environmental DNA is an emerging technique that has tremendous potential as a fisheries management tool. It however, has many limitations. This project is developing and testing this novel method for biomonitoring and filling in some of the major gaps in understanding that currently make interpretation of the results difficult or ambiguous. As this becomes a more widespread monitoring tool it will be essential that research has been done to support interpretation of the results so that the data are reliable and sound management decisions can be made.

Staff at OMNRF look forward to our continued participation in this project. Our support and involvement includes: provision of historical data; participation in meetings; expert advice; assisting with sampling and site visits; and review of draft documents. These contributions to the project have an estimated in- kind value of $4,500.00.

Regards,

Ken Cornelisse
Partnership Specialist
519-826-6849
ken.cornelisse@ontario.ca

400 Clyde Road, P.O. Box 729 Cambridge, ON N1R 5W6

**Phone:** 519.621.2761 **Toll free:** 866.900.4722 **Fax:** 519.621.4844 **Online:** www.grandriver.ca

September 12, 2019

Mark R. Servos, Professor

Canada Research Chair in Water Quality Protection

Department of Biology, University of Waterloo

200 University Ave. West

Waterloo, ON, N2L 3G1

RE: Global Water Futures research proposal: John P. Giesy et al. Next generation solutions to ensure healthy water resources for future generations.

The Grand River Conservation Authority (GRCA) manages water and other natural resources on behalf of 39 municipalities in Ontario and close to one million residents. The Grand River Conservation Authority has a tradition of watershed-based, integrated water management spanning more than 80 years.

The GRCA and watershed partners recently updated the Grand River watershed's Water Management Plan (WMP), which guides actions for the next 20+ years to achieve the four water management goals. One key goal is to improve water quality and river health. Prof. Servos played a key role in the development of the plan as a member of the Science Advisory Committee and he has had a very active and engaged research program in the Grand River for many years. His work has been contributing to a better understanding of the river's ecological processes as well as assessing how remedial actions improve the ecosystem health and resiliency.

The application of environmental DNA (eDNA) to support watershed bioassessments is an exciting new development and will advance our understanding of river health and provide water managers with tools to understand the species composition of the rivers in the watershed. The extension of the research in the Grand River will undoubtedly support the actions in the Water Management Plan and help to achieve its' goals.

The Grand River watershed continues to be under stress from population growth and urban development. New tracks of land are being annexed into urban areas with some of these areas having sensitive aquatic habitats. Many of the existing biomonitoring activities are time- and resource-intensive and yield limited information. New, innovative biomonitoring techniques such as eDNA will advance our abilities to provide timely information so that these lands can be proactively protected.

The original research focus on brook trout in small cold water streams was very timely. Ontario has been evaluating the apparent decline of Brook Trout populations in Ontario with the intention to

establish whether a broader management or policy approach for protecting and managing brook trout and their habitat in the province is needed. Biomonitoring using eDNA in the first phase of the project was demonstrated to be very sensitive and reliable for detection of brook trout in several small streams in the Grand River watershed (e.g. Washington, Blair, Bauman). However, as expected it remains difficult to directly assess fish abundance because of the poor understanding of the fate of eDNA in natural environments. Studies in the laboratory as well as controlled studies in the field have explored how eDNA degrades in the environment and is helping to create a framework for interpreting the in-stream results. There are many organizations looking to use eDNA in various studies and monitoring plans and the current research greatly enhances our organization's ability to interpret the results. There is now an opportunity to test and apply eDNA techniques in various sub-watersheds in the Grand River and this work will have implications for application across Ontario which could expedite protection of key fish species and their habitat.

The GRCA co-chairs the Grand River Fisheries Management Plan Implementation Committee that is responsible for implementing the best-bets for fishery management in the watershed. The members of this Committee have a wealth of information and expertise in the watershed and GRCA can help to facilitate the knowledge transfer among these stakeholders and the research team. Prof. Servos has been a very active member of this group and updates the committee on the results of the project at each meeting (several times per year). In addition, he has requested the opportunity to give formal presentations on this work to the committee, as well as to other interested parties in the watershed (e.g. environmental interest groups such as Trout Unlimited, Brant Rod and Gun Club Conference).

GRCA has a long history of supporting research as it leads to effective water management. This research proposal has tremendous potential to inform water and watershed management activities. The team has demonstrated a keen interest in knowledge mobilization and technology transfer in direct collaboration and support of the GRCA initiatives. They have applied the techniques in several sub-watersheds in the Grand River and coordinated work with GRCA staff in the past to allow added value to both the research and Authority's activities. The team is proposing to apply these emerging techniques in several additional streams (sub-watersheds) that are of direct interest to the GRCA (e.g. Bauman, Mill, McKenzie Creek) to test additional questions about the detection and assessment of aquatic communities. GRCA staff will continue to advise the research team, participate in meetings and provide information and data on fish populations, hydrology and water management as an in- kind contribution (estimated amount of $10K annually). I look forward to continuing to work with this high-caliber team of researchers.

Sincerely,

Crystal Allan

Supervisor, Natural Heritage

iisd|ela
experimental lakes area

September 5, 2019

Prof. John P. Giesy
Principal Investigator
Global Water Futures, University of Saskatchewan
44 Campus Drive, Saskatoon, SK S7N 5B3

Dear John,

We are pleased to provide this letter of support to accompany the joint application led by Drs. John Giesy (University of Saskatchewan) and Mark Servos (University of Waterloo) for a Global Water Futures program grant. Their proposal, entitled *Next generation solutions to ensure healthy water resources for future generations* fits closely with the mandate and current research interests and direction of the IISD-ELA, which is a whole ecosystem aquatic research program based at a facility located in northwestern Ontario (https://www.iisd.org/ela/about). Our mandate is to better understand human impacts on the environment and to influence the development of improved environmental policies and best practices. From 1968 until 2014, operation of the ELA facility and research program was the responsibility of the federal government; in April 2014, the program was transferred to the International Institute for Sustainable Development (IISD), a policy research group with its head office in Winnipeg and other offices in Ottawa, Geneva, and New York. IISD conducts extensive work on policy related to sustainable development including climate change and water in many countries around the world. IISD-ELA is now a not-for-profit, charitable organization.

Since 1968, ELA has been the site of multiple whole-ecosystem manipulations, mesocosm experiments, and long-term studies on the effects of human activities on the environment, especially freshwater lakes. Research from ELA has had a global influence on policies related to eutrophication and harmful algal blooms, acidification, the effects of water regulation, aquaculture, and contaminants (including whole-lake additions of mercury, artificial estrogen, and cadmium). The high impact of ELA research is largely because of our globally unique ability to undertake ecosystem manipulations, including additions of contaminants and chemicals to the environment. This ability has been recognized in unique legislation both at the provincial and federal level (https://www.ontario.ca/laws/regulation/140060; http://laws-lois.justice.gc.ca/eng/regulations/SOR-2014-95/page-1.html).

IISD-ELA is committed to supporting the proposed program in several ways. First, we are committed to supporting the experimental component of the program. This will be accomplished by performing controlled studies in mesocosms deployed within a lake to examine the fate and detection of eDNA with different species, under different conditions, and to test/apply these tools in the environment. IISD-ELA has an extensive record and vast expertise conducting studies in mesocosms, which are impermeable curtains suspended from floating collars and sealed to the sediments of a lake. Mesocosms, also called limnocorrals, allow the enclosed aquatic ecosystem to be characterized and manipulated as a model for lakes in which they are deployed. Mesocosms will be useful for these studies because we can control and precisely enumerate aquatic organisms (e.g. fish, benthic macroinvertebrates, phytoplankton, zooplankton) within the enclosures, collect water and sediment and isolate eDNA and then contrast this with samples from mesocosms where the community has been deliberately manipulated to be different. Sampling could be conducted over several seasons, across density gradients and eventually among multiple lakes and environments. In the longer term, IISD-ELA is interested in the application of eDNA

to identification of threatened or invasive species, habitat use/selection by target species, and even community assessments. IISD-ELA has several ongoing research projects and established population assessments that would be excellent opportunities to examine the utility of eDNA for delineating differences among aquatic systems.

IISD-ELA will also provide access to our 48-year database on meteorology, hydrology, water chemistry, and the food webs of ELA lakes to provide a freshwater context for the eDNA studies proposed by Drs. Giesy and Servos (estimated value of >$10,000). IISD-ELA will also provide in-kind contributions of at least $40,000 to the program, which includes time spent by Dr. Palace (4 wks/yr * $2800/wk * 3 yrs; total $33,600), IISD-ELA's Head Research Scientist, to attend planning meetings, analyze and assess data and contribute to manuscript writing and presentations of research results at conferences and community workshops. We will also contribute per diem expenses of IISD-ELA staff at the field station while working on the project ($16,000). Please note that per diem costs for NSERC supported researchers included in the project are out-of-pocket expenses for ELA and cover the room and board of researchers at the facility. IISD-ELA derives no financial benefits from these per diems and they are, in fact, heavily subsidized through our financial support from the Government of Ontario. Finally, we will afford access to our field research station to train graduate students and post-doctoral fellows and to perform studies in the 58 lakes that comprise our freshwater research station. Our investment reflects the importance we place on this research and we look forward to working with Drs. Giesy and Servos and to the results of this collaboration.

All manipulations at ELA, including the experiments proposed by this collaborative effort, must obtain approval from the IISD-ELA Research Advisory Board, which includes members from the Province of Ontario and several highly-esteemed external scientists. IISD-ELA will actively work with the study team to seek the necessary approvals for the proposed project.

In closing, we would like to emphasize the IISD-ELA's strong support for this proposal, which would enable all partners to achieve jointly well beyond the sum of their individual capabilities and produce great benefits for the Canadian freshwater environment.

Yours truly,

Matt McCandless
Executive Director
IISD Experimental Lakes Area

**Orano Canada Inc.**

817 45th Street West
Saskatoon SK S7L 5X2
Tel.: +1 (306) 343-4500

September 24, 2019

John P Giesy, Ph.D.
Professor & Canada Research Chair in Environmental Toxicology
Dept. Veterinary Biomedical Sciences
Toxicology Program Faculty, Toxicology Centre
University of Saskatchewan
44 Campus Drive
Saskatoon SK S7N 5B3

Dear Prof. Giesy:

**Re: Letter of continued support of the eDNA Research Initiative under the Global Water Futures Program**

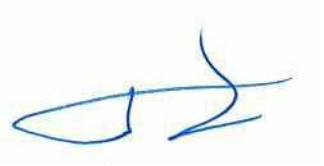
It is our pleasure to write this letter outlining Orano's (Orano Canada Inc.; formerly AREVA Resources Canada) continued support of the eDNA technologies research program, which shows promise to improve biomonitoring of Canada's aquatic ecosystems. Currently, the requirements of extensive environmental field monitoring studies (i.e. surveys of fish and benthic invertebrate communities) under the Metal and Diamond Mining Effluent Regulations (MDMER) are time, labour and resource intensive, and have a level of uncertainty that could be improved, should the objectives of these research initiatives be achieved.

The eDNA research program applies emerging and potentially transformative technologies in biological sciences, and provides the potential to address the needs of diverse end-users to advance and improve biomonitoring of Canada's aquatic ecosystems, and in that way support the continued sustainable development of Canada's resources. Specifically, if successful, the technologies developed by this research (e.g. eDNA technologies to replace current effluent and aquatic community monitoring approaches) could provide more efficient, objective, economic and ethical tools for the resource sector to fulfil our regulatory compliance requirements. Implementation of such alternative testing and monitoring methods, should their development be successful, could result in substantial cost savings, and contribute to better informed and more transparent decision-making in support of the continued sustainable development of Canada's resources.

The successful completion of the eDNA research is of great interest to Orano, and we look forward to our continued collaboration with yourself and Dr. Hecker and

his colleagues in an advisory role to support successful development of the tools under this program, as well as their applicability and usefulness to resource industries in the province.

Sincerely,



Vincent Laniece
Vice President
Safety, Environment and Engineering

September 6, 2019

To: Global Water Futures

Re: Letter of support to Global Water Futures - **John P. Giesy et al., "Next generation solutions to ensure healthy water resources for future generations".**

I am writing to express the support of Trout Unlimited Canada for the application to Global Water Futures entitled, "Next generation solutions to ensure healthy water resources for future generations".

Over the last 40+ years, Trout Unlimited Canada has focused our work, both nationally and locally on conservation and restoration of aquatic ecosystems and their native coldwater species. We are science-based, not-for-profit national conservation organization. We work both through our professional staff and our volunteer chapters on many watersheds across Canada, with some special focus on watersheds like the Grand River in southern Ontario where we have three of our chapters actively working on rehabilitation initiatives.

Trout Unlimited Canada (and its affiliated local Chapters) have been engaged in the research and have supported the researchers by providing advice/experience on the specific streams, helping to identify available data for various streams, and helping to collect samples. In addition, the research group has made presentations on their work at annual meetings as well as the Grand River Recreational Fisheries Implementation Committee.

Although only part way through the first phase of the project, it has made considerable progress. Brook trout in Washington Creek were assessed using three-pass electroshocking and the estimated fish abundance was compared to eDNA measurements at three sites sampled monthly over most of the year. A weak relationship was observed but interferences in the eDNA analysis was determined to be important. Lab and field experiments examined the fate and degradation rates of the DNA and this information will be very useful in interpreting eDNA in streams. EDNA is a promising tool although considerably more work is needed to better understand the fate and therefore understand the relations between fish abundance and eDNA response. Initial work was also done in Bauman Creek and metabarcoding applied to assess the fish community. This is showing great promise for bioassessment of fish and amphibian communities.

Trout Unlimited Canada sees eDNA as an emerging technology that may be extremely useful in managing fish populations and habitat. It, however, still is difficult to interpret in the context of biomonitoring. The work being proposed will greatly advance this tool so that it can be more widely and reliably applied in studies and used in fisheries management.

**Trout Unlimited Canada**
(519) 763-0888 1-800-909-6040
Unit 1-27 Woodlawn Rd W, Guelph ON N1H 1G8

As a not-for-profit, our financial resources are always limited, but we are willing and interested in participating with this initiative by providing support through participating in meetings, providing advice and where possible, assistance with some of the data collection on watersheds of interest to us. This would equate to an in-kind support ranging from $2,000 – 3,500 per year.

In summary, with limited funding for bio-assessment and monitoring, the development of new techniques and technologies to identify and provide early warning systems of ecosystem change would be extremely valuable to conservation organizations, whether governmental or NGO. With limited funding, any new technologies that provide sound information at reasonable levels of effort and efficiency will be essential in the future. We believe that this proposed applied research initiative would move us all a long way down that road.

Yours sincerely,

Alex Meeker, Ontario Provincial Biologist
Trout Unlimited Canada

**Trout Unlimited Canada**
(519) 763-0888 1-800-909-6040
Unit 1-27 Woodlawn Rd W, Guelph ON N1H 1G8

Trout Unlimited Canada

To *conserve*, *protect* and *restore* Canada's freshwater ecosystems and cold water resources for current and future generations.

September 3, 2019

To: Prof. Mark Servos
University of Waterloo
200 University Ave W.
Waterloo, Ontario

**Subject:** Global Water Futures proposal, Next Generation Solutions to Ensure Healthy Water Resources for Future Generations

The Middle Grand Chapter of Trout Unlimited Canada (TUC) is a group of community volunteers dedicated to improving water quality and aquatic habitat in the middle portion of southern Ontario's Grand River watershed, including the Nith River. We work in partnership with watershed residents, agencies, educational institutions, and other non-government organizations to conserve, protect and restore freshwater ecosystems in the Middle Grand River watershed in Southern Ontario.

We previously supported the first phase of the GWF project that has advanced the development and application of eDNA techniques for biomonitoring. I am writing this letter in support the second phase of this research program. The members of the Middle Grand TUC chapter would gladly provide logistical support, experience/advice and data we have collected, to ensure that this very worthwhile initiative is successful. Our chapter is community based and intimately familiar with many of the streams in southern Ontario that will be included in this research. We have established contacts with landowners in the area and have collected monitoring data using traditional aquatic survey techniques (e.g. electrofishing) for a number of years. We see many opportunities to develop new synergies as we work in partnership with the research community associated with this project.

During the initial research in small streams, including Washington Creek, the research described the trout population using repeated pass electrofishing and related this to the eDNA measures in water samples over the entire year. This information helps to validate the eDNA approach as well as support a better understanding of the distribution of the fish population. The studies have better defined the fate of DNA which will help to better interpret eDNA results in streams. As eDNA becomes a more common approach a better framework for interpretation and validity of results will be needed. The proposed research if addressing this need, while add the same time contributing to a better understanding of fish populations in our local streams. The Middle Grand Chapter of Trout Unlimited Canada has appreciated the research team presenting their results at our annual conference and having numerous discussions about the research. We look forward to continuing to contribute to this important initiative.

Please feel free to contact me at 226-821-1245 or lhalyk1837@gmail.com if you have any questions regarding our support of this very important initiative.

Sincerely,

Larry Halyk, M.Sc. President
Middle Grand Chapter Trout Unlimited Canada
c/o 43 Jason Dr. Guelph, ON, N1H 6J2

FRIENDS OF THE GRAND RIVER

Prof. Mark Servos
Dept. of Biology, University of Waterloo
200 University Ave. West
Waterloo, Ontario

19-Aug-24

Re: Grant application for the team led by J.P. Giesy et al., Next generation solutions to ensure healthy water resources for future generations.

Dear Sir or Madam:

The Friends of the Grand River (FOGR) is a charitable, non-profit stewardship group operating on the Grand River (a designated Canadian Heritage River) in southern Ontario. Our organization works on projects to restore and enhance river health, recreational fisheries and allied river resources. We work in partnership with other NGO groups, as well as municipal, provincial, and federal agencies to leverage volunteer support in a cost-effective manner on local projects benefiting the river resources. We keep our members informed on the improvements and attempt to present a strong unified voice to parties who may be developing policies and projects which affect the Grand River watershed by being part of the decision making process, and in the development of policies and projects which affect the Grand River watershed.

We enthusiastically supported the research in the original proposal and have seen it progress as it has been reported at the Grand River Recreational Fisheries Implementation Committee and other venues. The researchers regularly reach out to various groups for advice and support through this group.

The FOGR membership believes that the continued research has great potential for use in monitoring of invasive species, species-a-risk, and biological community change, as it relates to best scientific management of the Grand River. The research so far has enhanced the understanding of how fish populations move in small streams and how eDNA can be applied to detect their presence and even potentially heir health (e.g. RNA). Being able to more reliably understand and interpret eDNA monitoring data will be critical for supporting its future use in management decisions. We think that this research in the Grand River sub-watersheds will make significant steps in that direction.

FOGR has previously undertaken extensive river and stream monitoring projects and can share historical data and advice on future field studies to validate the applications of this important research. As the project proceeds, FOGR looks forward to contributing in-kind support such as providing advice and review, participating in collecting samples, and supporting educational (such as the streamside chats that we have done with graduate students related to this program) and supporting knowledge exchange among our members.

Sincerely,

Rob Voisin
Board Chair,
on behalf of the Board of Directors,
Friends of the Grand River

# ONTARIO FEDERATION OF ANGLERS & HUNTERS

P.O. Box 2800, 4601 Guthrie Drive, Peterborough, Ontario K9J 8L5
Phone: (705) 748.6324 • Fax: (705) 748.9577 • Visit: www.ofah.org • Email: ofah@ofah.org

Ontario Conservation Centre


September 11, 2019

To Whom It May Concern:

Subject: Regarding the research proposal: Next generation solutions to ensure healthy water resources for future generations (Giesy, Servos, et al.)

The Ontario Federation of Anglers and Hunters (OFAH) is Ontario's largest, non-profit, conservation-based organization, representing 100,000 members, subscribers and supporters, and 740 member clubs. Our organization is heavily invested in the conservation of aquatic resources and as such, we are supportive of the further development of technologies that will allow for more accurate and complete bioassessment of aquatic environments. The OFAH is directly involved in preventing and controlling the spread of invasive species in Ontario through our Invasive Species Awareness Program; a long-standing partnership with the Ministry of Natural Resources and Forestry.

The use of Environmental DNA (eDNA) technology is an emerging and powerful tool that is critical to controlling the spread of aquatic invasive species through early detection. In addition, eDNA metabarcoding using advanced sequencing technologies has many potential applications including biodiversity assessments. There are limitations for the practical implementation of eDNA in resource management, however, research is evolving quickly towards its widespread use. This research proposal is addressing some uncertainties and gaps related to the interpretation of eDNA by doing systematic comparisons in well-studied and characterized sub-watersheds in the Grand River (as well as other sites across Canada).

The OFAH continues to be fully supportive of the refinement of these technologies and applications to enhance their utility in resource management. This research is contributing to the knowledge and understanding of eDNA and its application by recognizing technological limitations, as well as unique opportunities. Using eDNA in combination with other tools can enhance our ability to manage fisheries resources and aquatic habitats.

The OFAH is a grassroots organization that is composed of individual volunteers and clubs that represent a wealth of local and historical knowledge pertaining to our aquatic resources. This research group has established connections to people in local clubs with an interest in participating in discussions and receiving advice on the development of this research. These connections are an effective means to transfer knowledge to people at the grassroots of fisheries management in Ontario. We have appreciated the group distribution of newsletters and research updates, as well as participation and presentations at local watershed committees and NGO meetings. In addition, OFAH members have participated in education, outreach and awareness activities such as training courses for graduate students. We anticipate that this productive exchange of ideas and information will continue to benefit both parties.

Yours in Conservation,

A. Weir

Adam Weir
Fisheries Biologist

September 4, 2019

Barbara A. Katzenback, Assistant Professor
Department of Biology, University of Waterloo
200 University Ave West
Waterloo ON, N2L 3G1

**RE: Global Water Futures renewal: John P. Giesy et al., Next generation solutions to ensure healthy water resources for future generations.**

This letter is to confirm our past and continued support of the research proposal entitled "Next generation solutions to ensure healthy water resources for future generations" that is being submitted for renewal to Global Water Futures.

The ***rare* Charitable Research Reserve** (***rare***) is an urban land trust and environmental institute located in the Waterloo Region/Wellington area of Southern Ontario, with its first four properties protecting almost 1,000 acres of highly sensitive lands. The goal of ***rare*** is to preserve its sites and their ecosystems intact in perpetuity, for the community to enjoy in their natural states. Through sustainable management, sensitive lands and research sites are protected while the public can enjoy 8 km of trails, extensive community gardens and regular interpretive events. While ***rare's*** goal is to steward its sites and ecosystems intact in perpetuity, it also promotes the lands as a living laboratory for research — including in-house monitoring programs and partnerships with other institutions, community-based scientists, artists and Indigenous Peoples. Research then informs restoration practices and education programs through a *Chain of Learning* that reaches even the youngest learners in a program called *Every Child Outdoors* (ECO), a model of active, hands-on, problem-based environmental learning, driven by inquiry in the out-of-doors.

The research funded by this grant has potential to increase the baseline monitoring capacities of our organization by validating cutting edge monitoring techniques for local use. The development of environmental DNA (eDNA) technologies for fish and amphibian species in Southern Ontario will be important for future use in monitoring species that reside in waterbodies that currently pose technical challenges for traditional survey methods.

Research conducted to date has aligned with multiple ongoing projects at ***rare*** since 2018. Research focusing on environmental nucleic acids has complemented ongoing monitoring efforts for Brook Trout in reclaimed portions of Bauman Creek, increasing spatial and temporal survey coverage along the creek. Research focusing on vernal pools will complement ongoing amphibian monitoring efforts such as the Marsh Monitoring program and seasonal cover board surveys. As little research has investigated the seasonal variation in amphibian species present in vernal pools on ***rare*** property or sampling design needed to capture seasonal and spatial variation in vernal pool eDNA, this research will lay the foundation for future non-invasive monitoring programs.

The ***rare*** reserve has an established history of supporting research pertaining to effective management of lands, water and the species that reside therein, and ***rare*** has been a strong supporter of the students funded by this research. In 2018, ***rare*** awarded Heather Ikert, a PhD student supported under this grant, a $2000 award from the Ages Foundation Fellowship Program to support their research into environmental nucleic acids. Additionally, ***rare*** has awarded Nathanael Harper, a MSc student supported under this grant, the 2019 Ages Foundation Fellowship valued at $4000 to support their research in environmental DNA metabarcoding for biomonitoring of amphibian populations in vernal pools over a temporal and spatial scale.



1679 Blair Road
Cambridge, Ontario
Canada N3H 4R8

+1 519 650 9336 phone
+1 519 650 5923 fax

rare@raresites.org
www.raresites.org

Staff will continue to advise the research team, participate in meetings, provide background information and historical data, coordinate volunteers, manage site access and contribute survey-hours with an estimated in-kind value of $1500 annually. We look forward to continuing our partnership with this high-calibre team of researchers.

Sincerely,

Jenna Quinn
Program Scientist – Research Priorities, Partnerships, & Monitoring
***rare* Charitable Research Reserve**
519-650-9336 x111
Jenna.Quinn@raresites.org
1679 Blair Road, Cambridge ON, N3H 4R8

ONTARIO WATER CONSORTIUM
LE CONSORTIUM POUR L'EAU DE L'ONTARIO

26 August 2019

Dear Global Water Futures:

RE: Project Proposal: John P. Giesy et al., Next generation solutions to ensure healthy water resources for future generations

On behalf of the Ontario Water Consortium (OWC), I am pleased to provide this letter in support of the following project proposal: John P. Giesy et al., Next generation solutions to ensure healthy water resources for future generations.

The OWC is a project of ten post-secondary institutions led by the University of Waterloo. It was established in 2011 with funding from the Federal Economic Development Agency for Southern Ontario (FedDev) and the province of Ontario, with a matching corporate contribution from IBM Canada and significant financial and in-kind support from other partners. With this investment of over $60 million, we have created a suite of unique facilities for water technology research, development and demonstration.

OWC is a client-focused portal that helps water technology companies access these facilities and connect to a critical mass of research expertise across the partner post-secondary institutions to commercialize water technologies. In 2016, with an additional $12 million in support from FedDev, OWC launched the Advancing Water Technologies Program (AWT) to advance clean water-related technologies. AWT is a catalyst for business-led collaborations in the water sector, designed to help Ontario water technology companies develop and demonstrate water technologies for successful introduction to the market. AWT provides financial contributions to industry-led research and development projects in the water sector that will result in the successful commercialization of globally-competitive and market-ready products and services. It also works closely with government and other organizations to support the adoption of innovative approaches to water treatment, monitoring and management.

The goal of the project proposal referenced above, a collaboration between the University of Saskatchewan, University of Waterloo and various water organizations, is to develop and apply emerging technologies to environmental assessment/monitoring including advanced chemical and biological techniques. OWC will be pleased to collaborate and support the project's use of key elements of the OWC platform to conduct its research. Equipment and infrastructure related to the OWC Analytical and Ecotoxicology nodes and sampling platforms have been utilized extensively in the first phase and will continue. The OWC Watersheds node monitoring infrastructure in Alder Creek and related data platform (including historical or current data sets)

ONTARIO WATER CONSORTIUM
LE CONSORTIUM POUR L'EAU DE L'ONTARIO

are also being integrated into the current research program and creating an opportunity to link biological and hydrological information to better understand the fate of eDNA in streams. The project will continue to use this infrastructure and access data to support the next phase. OWC is proud to be able to provide access to this unique infrastructure going forward.

In closing, OWC strongly supports this project proposal as it is an opportunity to expand its collaboration and impact on water research. I look forward to continued interaction with all involved organizations and researchers.

Sincerely,

Brenda Lucas, Executive Director
Ontario Water Consortium

Sept 10, 2019

**John P. Giesy, Ph.D.**
Professor & Canada Research Chair in Environmental Toxicology
Dept. Veterinary Biomedical Sciences
Toxicology Program Faculty, Toxicology Centre
University of Saskatchewan
44 Campus Drive
Saskatoon SK S7N 5B3, Canada

Dear John:
I am very pleased to know our collaborate project "**Next generation solutions to ensure healthy water resources for future generations**" have been gone exceptionally well and now moved to a new phase under the funding support by the Global Institute for Water Security at the University of Saskatchewan.

I think we have made significant progress over the last few years. Those results have resulted in some excellent joint publications. So I am keen to be able to continue to collaborate with you and all my colleagues at the Toxicology Centre at the U of S. We are willing to share technologies, expertise and data. Having your Ph.D. student Abby visit with us this summer will be an excellent start and then having my former Ph.D. student, Dr. Yuwei Xie join your team as a post doc will certainly help bridge the distance between our labs. He is excellent and very keen to have an opportunity to work with you and experience life in Canada.

As you know, since you have been working closely on our projects here, we have all of the equipment necessary to conduct joint and companion projects. We also have significant funding to support students and conduct projects. During our collaborations I expect that you will be returning to Nanjing on a regular basis and we will be sending students to work with you as well. Through this collaboration, we can take on some of the most pressing issues in the development of e-DNA into a useful technique that is used routinely. I have told our colleague and friend Prof. Hongxai Yu, at the Jiangsu Province Environment Agency, with whom we have been working closely. She is excited by the prospect that you will have access to funding in Canada to collaborate with us. She will send a separate letter supporting the collaboration directly to you.

As for leveraging your funding, we have the following project where our collaboration is highly relevant:

*2017-2020, National Water Pollution Control and Treatment Project of China (Project 2017ZX07602-002). "Key technologies of ecological control and water quality improvement of*

*the heavily polluted rivers in Huai Riverine basin". (¥3,800,000)*

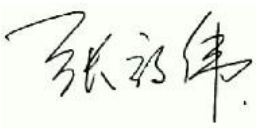
*2018-2021 National Water Pollution Control and Treatment Project of China (Project 2018ZX07208-002). "Technical Integration and Operational Operation of Biomonitoring in Water Eco-environmental Functional Zone of Tai Lake Basin". (¥14,800,000)*

In total, the funding we have in the area of ecogenomics is approximately $ 3,600,000 CAD. While we cannot provide cash support of your programs in China, the funded projects we have here in china will certainly be useful in addressing issue relative to establishing Meta-barcoding as a routine tool for monitoring of biodiversity in aquatic systems. Methods developed and data generated on our joint projects here in China will certainly compliment what you can do in Canada. Besides, we can support the studies done by your students when they are here in China if you can support the efforts of our students when they are working with you in Canada.

John, I am keen to continue working with you and your team. We have been extraordinarily productive since we started working together in 2003. I look forward to our future collaborations and continued productivity, especially in training of students.

Sincerely,

Sincerely yours,



**Xiaowei Zhang, PhD, Prof.**

Associate Director, National Engineering Research Center for Organic Pollution Control and Resource Reuse

School of the Environment
Nanjing University
163, Xianlin Avenue.
Nanjing, Jiangsu, 210023 China

Tel: (86) 25-8968 0623
Cel: (86) 151 5188 1287
http://hjxy.nju.edu.cn/files/faculty/zhangxw.htm

MUNI | RECETOX

John P. Giesy, Ph.D.
Professor & Canada Research Chair in Environmental Toxicology
Dept. Veterinary Biomedical Sciences
Toxicology Program Faculty, Toxicology Centre
University of Saskatchewan
44 Campus Drive
Saskatoon SK S7N 5B3, Canada

Brno, September 9, 2019

Dear John,

We are very glad to continue to collaborate with your research team on your project entitled: Next generation solutions to ensure healthy water resources for future generations, funded by the Global Water Futures program and administered by the Global Institute for Water Security at the University of Saskatchewan.

During our collaboration on this project we have built on our long-term cooperation, which lead to many joined interesting research results and scientific papers. As you know from your previous visits and collaboration with us, we have up-to-date equipment for wide spectrum of comprehensive chemical and toxicological analyses as well as established unit for microbiome and eDNA analyses, ready to be used for various collaborative projects. We appreciate you providing us with SOPs and training to move those joint projects along. In particular, I appreciated the opportunity to spend two weeks in your laboratory learning eDNA techniques and specifically the trip to the ELA to help collect eDNA samples was outstanding.

We really appreciate your involvement as a Scientific Board member of RECETOX, where together with other experts you are providing expert feedback on our research and management strategies. Your great achievements in the scientific field and long-term productive collaboration with our university actually lead to your selection for the award of honorary degree Doctor Honoris Causa in the field of Environmental Sciences by our university, which will be awarded this December.

During our collaboration on this project we expect you will keep coming to RECETOX and also send some of your students and/or post docs to do collaborative research at our institute. We would also like to send post docs and students to work with you and your colleagues, especially if the ERASMUS+ program will continue to provide support for these stays as in previous years. The collaboration will be certainly strengthened by the Marie Currie project of our colleague Garry Codling with the project entitled: "Finding unknown endocrine disrupting compounds through target pull-down assay filtration, effect direct analysis and ultra-high resolution mass spectrometry for a

comprehensive efficient workflow" that he will be doing with you at the University of Saskatchewan, starting to work in your lab this fall and come back to RECETOX in two years. We think that eDNA technology is a very powerful technique, gaining a lot of attention with wide applications both for research but also regulatory purposes. In our current research we are developing the approaches and pipelines for using this technology and other omics namely towards characterization of phytoplankton (mostly cyanobacterial) water blooms and their production of bioactive compounds as well as for characterization of some little described aquatic communities, including samples from Antarctica. Here we are strongly building on our undergoing collaboration and know-how transfer that was also mediated during my visit and 5-month stay of our PhD student Marek Pipal at your institute as well as training and research stays of two students from your department (Alper James Alcaraz, Derek Green) at Masaryk University within last year. Mutual collaboration with your research group and the mentioned project are definitely beneficial for both institutions in the Czech Republic and Canada.

As for leveraging your funding, we have the following on-going projects where our collaboration is highly relevant:

2017 - 2020: EU H2020 ITN 722493 - Natural Toxins and Drinking Water Quality - From Source to Tap (€ 464840)

2018 - 2020: GAČR 18-15199S - Bioactive compounds from cyanobacteria affecting nuclear receptor signaling and vertebrate development (€ 200200)

2019 - 2023: EU H2020 825753 - Breaking down the wall between human health and environmental testing of endocrine disrupters: EndocRine Guideline Optimisation (€ 417875)

We also very much value our continuing joined research activities in other planned and proposed follow up projects, which align well with your research directions and would provide support for our further collaboration through exchanges of faculty and students.

We are looking forward to further collaboration with you and your research group.

Sincerely,

Klara Hilscherova, Ph.D., senior scientist

RECETOX, Masaryk University, Faculty of Science
Kamenice 753/5, pavilion A29, 625 00 Brno, Czech Republic

PHONE: +420 549 493 256
MOBILE: +420 776 741 900
E-MAIL: hilscherova@recetox.muni.cz WEB: www.recetox.muni.cz

FUNDACIÓN
MERI

Prof. Mark Servos
Department of Biology,
University of Waterloo
200 University Ave. W.
Waterloo, Ontario, N2A 3G1, Canada

*Re: Global Water Futures, Next Generation Solutions to Ensure Healthy Water Resources for Future Generations.*

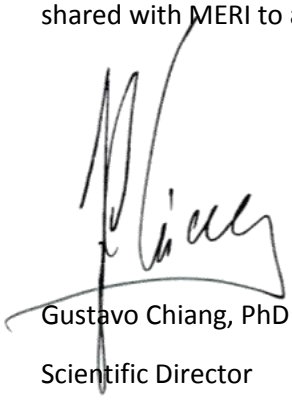
The Melimoyu Ecosystem Research Institute (MERI Foundation) has a vison that through a socially inclusive private initiative, it can inspire and promote the conservation of biodiversity, the natural and cultural heritage of northern Patagonia. The Melimoyu Nature Reserve was established in order to protect the and strengthen research and education for the conservation and sustainable management of the terrestrial, freshwater, marine and cultural heritage of northern Patagonia. The Melimoyu Nature Reserve is located at north of the region of Aysén, in the fjords of the continental coast, 300 km south of Puerto Montt. The imposing Melimoyu Volcano dominates its geography, 2,400 meters above sea level, and two fresh-water rivers cross the Nature Reserve from east to west, the Colonos and the Marchant, flowing into the Melimoyu Bay (more than 16 thousand hectares of native forest and seven kilometers coastal marine edge). The Melimoyu Nature Reserve was established by the MERI, a private non-profit organization, in order to investigate, interpret, educate and ensure the conservation of pristine ecosystems and terrestrial and marine ecological diversity of the Melimoyu Nature Reserve and its surroundings.

The aquatic ecosystems of the two remote and pristine rivers in the Melimoyu Nature Reserve have only started to be scientifically documented since the establishment of the reserve in 2012, with research projects related to fish and aquatic health. Due to the isolated nature of the area, we initiated a pilot study with Dr. Servos in 2018 to determine if environmental DNA (eDNA) could be used to detect the invasive brown trout in this ecosystem. The preliminary results were very promising and were validated with electrofishing. We now wish to further this research to look at the fish communities in this system using metabarcoding and extending it spatially. This work would help to better define the biodiversity of these isolated river systems and focus our scientific efforts to define, understand and protect this unique ecosystem.

Over the course of the project, MERI staff would facilitate the visit of researchers from the GWF project to visit the Melimoyu Nature Reserve. With our team we would use a jet boat and electrofishing equipment to help collect water and fish samples from the river and bay environments. We would

Melimoyu Ecosystem Research Institute
Av. Kennedy lateral 5682
Vitacura-Santiago, Chile
+56 2 29535192

provide access to our lab facilities for processing and preservation of samples on-site. The researchers would be able to stay at the remote research station (at established rates for research collaborators). This represents an important contribution to the research initiative. We would ask that all the data be shared with MERI to advance our efforts to promote conservation in this important region.



Gustavo Chiang, PhD

Scientific Director

Melimoyu Ecosystem Research Institute

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