UNIVERSITY of GUELPH

Pathway to Increase Standards and Competency of eDNA Surveys (PISCeS)
A National Workshop on Environmental DNA

Oral Presentation Abstracts

What if… How else… Integrating environmental DNA into applied ecology

Jennifer Petruniak
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As global attention on biodiversity increases in response to rising concerns related to climate change, invasive species and species at risk, academia will be at the forefront of developing novel ways to utilize ever-advancing DNA sequencing technology to monitor biodiversity using environmental DNA. At the same time, the pace of development is increasing and industries are faced with mounting pressures to operate in an environmentally sustainable way. This pressure is linked to social license, meeting the requirements of environmental legislation, or both.

For industry, the challenge of pairing environmental protection with survey timelines, taxonomic expertise, surveys costs and meeting overall environmental regulatory approval requirements can be challenging and create uncertainty.

Environmental DNA is an important step in advancing our abilities to collect ecological data. An integrated partnership between academia, industry and government is crucial in evolving the use of this technology in meaningful ways. In this talk, examples will be provided from an industry perspective on where a bridge between academic development and government approvals can, and should, be built.

Multiple repositories of environmental DNA: triple source detection of the Endangered Jefferson salamander *Ambystoma jeffersonianum*.

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Environmental DNA (eDNA) is a component of potentially multiple environmental sources which can be used to determine the presence of target species. The most commonplace resource utilized as a source of eDNA in the pursuit of macrobial aquatic organisms is water, within which eDNA from aquatic organisms is found both in solution and as part of suspended material. However, many water bodies are ephemeral, narrowing the sampling opportunity of water-borne detection of species whose presence in these vernal ecosystems is also transient. Ambystomid salamanders utilize vernal pools of southern Ontario to lay their eggs, sustain larvae and to house developing adults, which need to attain maturity within the timespan of the pool's lifetime. The window of detection is extremely short, and variable from year-to-year, and is particularly acute for the most imperilled of Ontario's caudata species, the Jefferson salamander *Ambystoma jeffersonianum* (SARA, schedule 1: Endangered). *A. jeffersonianum* cannot morphologically be distinguished from the more common unixsexual Ambystomids making conventional identification impossible. We developed a species-specific eDNA assay to test whether *A. jeffersonianum* could be detected from three vastly different repositories of eDNA: traditional water samples, aggregated dead/empty salamander egg matrix and desiccated vernal pool topsoil, in addition to implementing an expanded occupancy analysis to model sampling effort required for detection. Whilst detection was higher for the water samples, we succesfully detected *A. jeffersonianum* eDNA from all sources, suggesting out-of-season detection may be possible to determine important reproductive and nursery sites of this, and other, critically endangered species.

eDNA, a successful tool for biomonitoring Species-At-Risk and Aquatic Invasive Species in highly acidic aquatic environments

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Environmental DNA (eDNA) is an advantageous biomonitoring tool that allows the early detection of aquatic species at very low densities, which could be otherwise challenging using traditional techniques. eDNA surveys are essential for detecting and monitoring rare and elusive species using a non-invasive method and without direct observation. Additionally, eDNA surveys are valuable to mitigate the impact of biological invasions, facilitating prompt detections and improving eradication efforts. While the use of eDNA increases, there are still uncertainties about the effect of environmental variables, such as pH, upon DNA degradation and detection

probabilities. Here, we assess the ability of eDNA sampling to detect the presence/absence of one species-at-risk (Blanding's turtle) and two invasive aquatic species (Chain pickerel and Smallmouth bass) in Kejimkujic National Park and Historical site, where the aquatic system is highly acidic. We designed and validated a species-specific qPCR taqman assay for each of the three target species. Five replicates of 1L water samples were taken per sampling site (five sites for Blanding's turtle and chain pickerel, seven sites for smallmouth bass). Water filtration and eDNA extractions were performed on-site, while qPCR reactions were performed in the Lab. Despite the low water pH, our results show positive eDNA detections in almost all expected positive sites (except in one site for Blanding's turtle and chain pickerel). Additional detection was observed in sites where the presence of the target species was previously unknown. Our study supports the advantage of eDNA to detect species in low densities, and its value in management strategies and conservation biology.

Utilizing eDNA to detect SAR when others have failed: Redside Dace in Irvine Creek

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Irvine Creek, protected as regulated habitat for Redside Dace (*Clinostomus elongatus*), is running out of time. When AECOM applied for a permit under the Endangered Species Act (ESA) in early 2017 for a bridge replacement project over Irvine Creek it was revealed that Redside Dace had eluded capture since 2001 and was fast approaching the end of the 20-year regulatory protection window. Consultation with MNRF concluded that a presence/absence study was needed to determine if Redside Dace still inhabited Irvine Creek.

Various means of conventional and non-traditional sampling methods had historically been employed on Irvine Creek, including: seining, electrofishing, and even videography. Due to the limitations of site conditions, and sampling gear restrictions for up-listed SAR, it was evident that alternative means of sampling was necessary. Irvine Creek was a perfect candidate to implement eDNA. Working with the University of Guelph and Precision Biomonitoring we received approval from our client and MNRF granted the use of eDNA as an Overall Benefit Measure as part of the ESA permit.

Our presentation details the opportunity that generated eDNA for use as part of the regulatory permitting process, including: agency consultation, sample design (including amendments); QA/QC and sampling confidence, restrictions/limitations, preliminary results (pending agency approval), and potential implications to regulatory framework (inferred questions).

Regulatory considerations for eDNA: The good, the bad, and the ugly

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eDNA technology provides an entirely non-invasive means of conducting ecological surveys without physically harming organisms, particularly species-at-risk, and at a considerably lower sampling effort and cost. As with any emerging technology, substantial hurdles exist in securing regulator and stakeholder acceptance of eDNA as a valid and feasible alternative to traditional biodiversity studies employed for environmental assessments, industrial compliance monitoring, or restoration/remediation success criteria. Our presentation will employ case studies from contemporary industrial projects highlighting successful adoption of genomic biodiversity tools, projects that fell short, and a discussion of current barriers to regulatory uptake with suggestions for potential paths to future success.

Recent progress in the development and application of robust targeted eDNA detection methods: The BC experience

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Timely delivery of robust and accurate information is required to ensure effective and efficient environmental protection and management. Information pertaining to the distribution of aquatic and semi-aquatic native and invasive species can now often be most efficiently achieved *via* application of eDNA methods.

The credibility of eDNA survey methods depends on adequate methodological validation and verification; accurate results require rigour at multiple stages including field sampling, sample handling/processing, laboratory test design/validation/analysis, and test result reporting/interpretation. We have tackled these issues through promotion of a shared and transparent understanding of eDNA methodological considerations in all aspects of eDNA project design.

This presentation provides an overview of the activities undertaken to increase the standards and competency of eDNA surveys in western Canada. This includes the development of accepted eDNA field and laboratory standards by the BC Ministry of the Environment and Climate Change. Based upon experience of over 40 eDNA projects since 2014, particular focus has been on developing robust field and laboratory pipelines for consistent application in academic, government and commercial settings. Education and community-building has taken the form of a recent western Canada regional workshop and training courses provided through the Natural Resources Training Group.

This presentation discusses the strengths and limitations of eDNA using lessons learned through implementation and practice as a tool for addressing best practices and minimum data collection/reporting requirements to inform environmental management. The presentation also discusses critical considerations for further advancement of eDNA methods using a standardized approach to ensure rigour in environmental assessment practices.

Mapping invasive and native invertebrates in two great lakes tributaries using environmental DNA (eDNA) metabarcoding

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Aquatic invasive species (AIS) impact, and pose ongoing risks to native species and ecosystems including in the Laurentian Great Lakes (hereafter Great Lakes). We describe using massively-parallel high-throughput next-generation sequencing and metabarcoding of environmental DNA (eDNA) to determine distributions and potential AIS impacts across 86 sampling sites in two major Great Lakes tributaries (Grand, Sydenham Rivers, South Western Ontario, Canada). Sampling began near each river mouth and extended into upper-river reaches. We used a universal PCR primer set targeting CO1 and relatively strict parameters when BLASTing quality controlled sequenced amplicons against customized species-level reference databases composed of: established Great Lakes AIS; AIS likely to expand into Great Lakes basins; mollusks; chironomids; rotifers; and many crustaceans. AIS identified are likely the first such observances in these rivers and included: a freshwater jellyfish (*Craspedacusta sowerbyii*), two oligochaetes (*Branchiuria sowerbyi*, *Potamothrix moldaviensis*) and a copepod (*Skistodiaptomus pallidus*). Rare important native mollusks identified included the provincially endangered rayed bean (*Villosa fabalis*) and threatened mapleleaf mussel (*Quadrula quadrula*). Each river had overall different species compositions, for example, the Grand had 23 of 56 total species we identified, whereas we identified 42 of the same overall 56 taxa in the Sydenham River. Taxa presence also varied among sites within rivers. At sites in the mid-reaches of the Sydenham we observed overlapping occurrences of AIS and rare native mollusks. This locale presents opportunities for further: examinations of trophic interactions, determinations of mechanisms of previous AIS dispersals, efforts to limit AIS spread, and for characterizations of AIS impacts.

Evaluation of freshwater fish communities with eDNA metabarcoding: a quantitative perspective

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Accurate data on species distribution and abundance are critical for conservation and management of aquatic resources. Several inventory methods, such as gillnets survey, are widely used to estimate those parameters. However, gillnets can be invasive, costly in terms of material and human resources, may cause unwanted mortality in the fish communities studied as well as been subject to size and species selection bias. Environmental DNA (eDNA) analysis, which consists of detecting DNA traces released by species in their environment, could be used as a non-invasive, more accurate and less costly alternative or complementary than so-called method. In this

study, we evaluate the pros and cons between eDNA (eMetabarcoding) and gillnets for monitoring freshwater fish communities in terms of species richness, relative abundance and species abundance. Our main observations are : i) eDNA detected more species than gillnet; ii) detection sensitivity improves with the amount of filtered water; iii) sequence reads, qPCR, and catch per unit effort were correlated for the 3 main species. In sum, eMetabarcoding is a very valuable complementary, if not alternative tool for freshwater fish monitoring.

Primer validation for metabarcoding

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Metabarcoding has emerged as an excellent tool for assessing communities using environmental DNA, gut content samples or even DNA from preservation ethanol (specimen recovery). However, this PCR-based technique requires the selection of proper primers in order to reduce primer biases and to maximize taxa recovery.

We tested 21 different metabarcoding primer pairs using an insect mock community, containing 374 different specimens. Well designed primer pairs, with sufficient degeneracy did recover most taxa in almost identical proportions. Despite popular belief, using several primer sets did not improve taxon recovery. While we found several well performing primer sets, some showed substantial non-target amplification at lower temperatures. This is especially relevant for eDNA samples, where often only a few target molecules are present, compared to e.g. template DNA derived from bacteria or zooplankton. Here ribosomal markers might be advantageous, provided that a reference database can be generated in a reasonable timeframe.

Our study highlights that careful primer evaluation is essential for eDNA metabarcoding projects. While our study was carried out using an insect bulk sample, results will likely translate into DNA based macroinvertebrate monitoring in general.

A system for rapid eDNA detection of aquatic invasive species

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Environmental DNA (eDNA) detection of aquatic invasive species using PCR is a powerful new tool for resource managers, but laboratory results often take weeks to be produced which limits options for rapid management response. To circumvent laboratory delay, we combined a purpose-built eDNA filtration system (ANDe) with a field DNA extraction and handheld qPCR platform (Biomeme) to form a complete field eDNA sampling and detection process. A lab study involving serial dilution of New Zealand mudsnail eDNA was conducted to compare the detection capabilities of our field system with traditional bench qPCR. Two field validation studies were also conducted to determine if the on-site eDNA process can be used to map mudsnail eDNA distribution and quantify temporal fluctuations. Both platforms (Biomeme, bench qPCR) lost the ability to reliably detect mudsnail eDNA at the same dilution level (10^{-4}), with SQ values as low as 21 DNA copies/reaction. A strong relationship was observed between the average Cq values of the two platforms (slope = 1.101, intercept = - 1.816, $R^2 = 0.997$, $P < 0.001$). Of the 80 field samples collected, 44 (55%) tested positive for mudsnail eDNA with Biomeme, and results identified both spatial and temporal fluctuations in mudsnail eDNA/L. However, the PCR inhibition rate (no IPC amplification) with Biomeme was 28% on average for field samples, and up to 48% in the temporal dataset. With additional optimization of the DNA extraction process, the ANDe-Biomeme system has potential to be a rapid and highly effective detection/quantification tool for aquatic invasive species.

Environmental DNA educational resources for power generation and distribution

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Environmental DNA (eDNA) monitoring techniques, along with other genetics techniques, are becoming less expensive and more accurate, and are gaining greater acceptance in regulatory settings. These technologies have the potential to revolutionize the detection of rare and elusive species, identification of habitat use and preferences by those species, as well as supporting occupancy and use for restoration success. Such technologies are evolving rapidly with potentially broad applications to energy sector monitoring and compliance support, including monitoring and conservation of endangered and protected species. The Electric Power Research Institute (EPRI) has been working to educate and update its members on the state of the science in genetic monitoring and identification, trends and industry-relevant applications, and directions in eDNA science that may improve existing and future monitoring and compliance methods. This talk will discuss and highlight some of the published products, education resources and planned efforts that EPRI is providing for power companies, resource agencies, stakeholders and the public.

The Role of Standards in Supporting eDNA Innovation

Ana-Maria Tomlinson
CSA Group

All new and existing projects within a natural environment, particularly in the natural resources sector, must perform environmental assessments to show that their activities will not cause undue impact to biodiversity and the environment. Environmental stewardship requires timely, accurate information on the status of a given ecosystem and the species that occupy it. Environmental DNA, also referred to as eDNA, is a powerful, innovative method which provides a more cost-effective, less invasive and more efficient surveying technique for species identification compared to traditional assessment methods. Having been studied and perfected through research, this relatively novel tool faces the challenge of building and inspiring trust among users and regulators for wider acceptance and application.

Standards can help emerging technologies, such as eDNA, address such challenges by establishing a common set of requirements and setting performance expectations across a technical area. Standards define a common framework for emerging technologies, by outlining the required set of attributes of a product or service, and showcasing and encouraging the best practices available within an area of expertise.

As an accredited Standards Development Organization, CSA Group has close to 100 years of experience in the development of national, bi-national and international standards. This presentation will provide an overview of the accredited standard development process, and a detailed look into how standards can complement regulations, and help commercialize emerging technologies. Case studies of standards supporting emerging technologies and accelerating the pace of innovation will be explored, and a standards path for eDNA technologies will be presented.

Harnessing eDNA for resource management

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The primary uses of eDNA data, species detection and reducing uncertainty about their presence or absence, have clear applications for management agencies. Although increasingly used for monitoring for invasive species, there is a clear demand for documenting occurrences of rare or at-risk species, and uncertainties remain about how best to interpret and work with eDNA data. Currently, many of these latter applications are limited to scientific studies and are not readily adopted by management agencies. To move eDNA beyond local scientific studies and harness its potential for species management, findings must be reported and stored in a data repository that is readily accessible for management and planning decisions.

Sensitive information for occurrence of at-risk and highly valued species in Ontario is housed in the Ministry of Natural Resources and Forestry's Natural Heritage Information Centre (NHIC), which maintains spatial occurrence data for species of management interest. To ensure its utility, information stored in the NHIC database must include spatial information and meet data quality standards. Although these standards are well-established for specimen reporting and observational data, the indirect nature of eDNA detections poses some challenges for its widespread acceptance. Using Ontario case studies from Ontario as examples, we will discuss information challenges and needs relating to reporting eDNA observations, highlight some potential pitfalls, and suggest

solutions. Establishing standards for field sampling, testing, and metadata will help facilitate acceptance of eDNA data by management agencies.

One federal researcher's perspective on how to move towards harmonizing approaches in support of implementing eDNA surveys for decision-making

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Environmental DNA methods promise to provide substantial improvements to current methods of monitoring marine and freshwater environments for a multitude of applications including species at risk, invasive species, aquaculture impacts, and marine protected areas. However, many steps other than the development of the science itself need to be taken to capitalize on the potential for eDNA methods to contribute directly to decision-making. While it is well recognized that assays need to be tested before they are implemented for management, standardized guidelines are needed that outline minimum criteria for determining fitness-for-purpose of an eDNA assay. This would enable managers to do their own due diligence to ensure results they are interpreting are generated by defensible methods given their particular need. Similarly, guidelines for best practices are needed so managers can evaluate whether an assay is being implemented within laboratory conditions that are amenable for generating reliable eDNA results. Last, minimum reporting criteria are needed to ease communication and support optimal integration of eDNA data by managers into existing workflows to maximize improvements to desired outcomes over current monitoring methods. Strict methods standardization is not realistic for use across broad applications and would only bog us all down in the trying, whereas best practices guidelines and minimum criteria can be designed to be readily implementable across a range of eDNA applications. As such, they would directly support the natural progression towards the development of standardized protocols for particular assays as the science advances.

Poster Presentation Abstracts

Precision ecosystem health and environmental omics: Importance of reproducibility, data reliability, and comparability

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References to and applications of "precision health" for human and community well-being have surged in recent years. Genomics and other molecular diagnostic tools are currently transforming preventative health care, diagnostics and treatment, both for individuals and communities. Environmental DNA (eDNA) tools for distributional analyses of individual taxa as well as surveys of biological community richness and composition (based on metagenomics), along with toxicogenomic/metabolomic methods, are of significant interest for advancing "**precision ecosystem health**" monitoring and management as a much needed, transformative approach for knowledge acquisition and application. The role of environmental omics approaches in advancing precision ecosystem health monitoring and management, however, is currently undermined by insufficient focus on data reproducibility and cross-study / cross-survey data comparability, which is fundamentally important for cumulative effects monitoring and meta-analytical approaches. The rapid proliferation of eDNA and metagenomics studies in the published literature has resulted in a large number of compelling, highly useful advances, with each study addressing its stated intrinsic objectives with internal consistency and rigour. It is highly challenging, however, to compare conclusions and the supporting data across the hundreds of studies as a result of major methodological and interpretative differences. Among the issues is a systemic lack of concern about reproducibility and relative independence from researcher bias, cornerstones of all modern science. We define a provisional set of guiding principles - as a starting

point for dialogue and further improvements - for the conduct and interpretation of environmental omics studies conducive to more powerful cross-study comparisons, and ultimately advancing precision ecosystem health monitoring and managing.

Shrimp stomach contents as biodiversity capsules for fish biodiversity monitoring

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The detection of environmental DNA in either sediment or aqueous samples is rapidly gaining popularity for the monitoring of fish communities in space and time, especially since traditional fish surveys are invasive, costly, labour intensive and highly selective. Estuarine environments are a challenging environment for eDNA studies due to their extensive water movements, influencing eDNA migration, and their high concentration of organic material which requires a long water filtering process. Here, we evaluate the application of a novel, high-throughput DNA-based monitoring tool to assess fish diversity, based on the analysis of the gut contents of a generalist predator/scavenger, the European brown shrimp, *Crangon crangon*. Sediment and shrimp samples were collected from eight European estuaries and additional water samples and net surveys data were collected from two of those. DNA metabarcoding (using a 12S marker) was carried out to infer fish assemblage composition. We detected 20 teleost species using metabarcoding, which was twice as many species as recovered by traditional net surveys. This method has the advantage that the "sampled biodiversity" is naturally encapsulated in the gut of a shrimp, representing a significant way to streamline and by-pass many of the fastidious steps required to reduce degradation and contamination when sampling water or sediment. This is a fact that is often underemphasized in eDNA research. By comparing and interweaving trophic, environmental DNA and traditional survey-based techniques, we show that the DNA-assisted gut content analysis of a ubiquitous, easily accessible, generalist species may serve as a powerful, rapid and cost-effective tool for routine, large scale estuarine biodiversity monitoring.

An analysis of metadata reporting in freshwater environmental DNA research calls for development of best practice guidelines.

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The detection of environmental DNA (eDNA) is emerging as powerful biomonitoring tool in varied settings with a particular emphasis on freshwater systems. In order to assess data reporting criteria for eDNA studies in this domain, we conducted a literature review of 187 primary journal articles published between January 2014 and January 2018 that were focused on eDNA detection in fresh water. We highlight the fact that there is currently no standardized method for environmental DNA (eDNA) detection or harmonized framework for meta data reporting. Based on our review of the literature, we established a rubric that included reporting criteria relating to the categories of study objectives, experimental methods used to collect data and statistical methods employed for data analysis. We then scored each paper based on whether or not they reported on the key parameters of the rubric. We determined there were substantial inconsistencies among studies concerning meta data pertaining to environmental conditions at the time of sampling, when and where samples were collected and how they were preserved. This observation suggests that the development and use of standardized data reporting guidelines would benefit future studies and we provide meta data reporting recommendations for researchers wishing to conduct studies in the field of fresh water eDNA.

Comparative assessment of presence and abundance of potamodromous fishes using two-pass electrofishing and eDNA detection.

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In recent years, the use of environmental DNA (eDNA) has become a prevalent biomonitoring tool to infer presence of a target, but its future potential lies in an ability to accurately quantify the abundance and/or biomass of aquatic species in natural systems. However, few studies to date have demonstrated this potential compared to conventional methods currently in use (e.g.,

electrofishing). In this study we assess the power of targeted qPCR eDNA surveys to detect and quantify the abundance of two target species, the burbot *Lota lota* and the brook trout *Salvelinus fontinalis*, compared to conventional electrofishing, in a low productivity stream system at a gold mine in northern Ontario. Detection data was used to feed probabilistic models to infer the probability of detection of each species across the two methods, as well as site occupancy and the probability of detection per sampling event. Additionally, correlation-based analyses sought to disentangle whether site-specific abundance, as determined by capture data, could be predicted by the strength of the eDNA signal. The results confirmed that eDNA sampling is an effective tool for biomonitoring. However, our results from the occupancy analysis also suggest that factors such as environmental variables asscoiated with water quality should be carefully considered when using eDNA in aquatic species surveys and in the interpretation of results.

Enhancing the determination that absence of proof *is* proof of absence: Lower Seymour Conservation Reserve, Vancouver BC.

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The Lower Seymour Conservation Reserve (LSCR) in North Vancouver, British Columbia, is home to a diverse assemblage of amphibians, reptiles, fish, and mammals, many of which are protected federally through the *Species at Risk Act* (SARA). Among this assemblage are the SARA-listed Coastal Tailed Frog (*Ascaphus truei*), Red Legged Frog (*Rana aurora*), and Pacific Water Shrew (*Sorex bendirii*).

During a remediation program within the LSCR that included stripping contaminated soils, realigning ditches, and altering hydrology of a downstream wetland, AECOM implemented an eDNA analysis program to determine whether remediation works had potential to affect any of the above species. The client was reluctant at first to adopt the technique, but following extensive dialogue and in situ demonstrations, the client acknowledged the benefits of adopting this minimally invasive technique: using eDNA avoided the need for time-consuming and potentially lethal trapping of the target animals.

In collaboration with a laboratory in Southern California, AECOM collected numerous water samples within the project footprint and from surrounding areas, using peristaltic pumps and Sterivex filters. Laboratory analysis indicated the absence of all targetted species. This result, in combination with a detailed site assessment, obviated the need for an amphibian salvage survey, enabling the client to proceed more quickly with remediation works. The client has since adopted the eDNA sampling method as an in-house method for species presence/absence assessment throughout the conservation reserve.

Our presentation illustrates the advantages of potential adoption of eDNA sampling method as part of the effects assessment process. We outline the challenges in adopting this technology and the role of the scientist in explaining it, sample design and confidence, limitations, and opportunities for using the technique in various ways across conservation areas in presence/absence studies and habitat range delineation.

Examining the ecology of eDNA in riverine systems: A case study using imperilled freshwater mussels

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Freshwater mussels are important to healthy riverine habitats as they contribute to bottom up trophic dynamics and influence water quality. Mussel populations are declining due to habitat loss and degradation resulting from human activity. Fifteen out of 41 freshwater mussels species native to Ontario are endangered, threatened, or of special concern. Environmental DNA (eDNA) has risen to prominence as an effective molecular method for detecting organisms. The sensitivity of eDNA methods make it an appropriate choice for detecting species at risk (SAR), which can be present at low abundances and difficult to detect using conventional methods. eDNA sampling has negligible levels of environmental impact, compared to conventional detection techniques, this reduces the disturbance on SAR if they are present in the area. The ecology of eDNA in riverine systems is ambiguous in the current literature; maximum detection distances range from hundreds of meters to several kilometers. The purpose of this project is to optimize sampling designs for eDNA surveys in riverine systems and to model the detection probability and habitat occupancy of a target species, *Lampsilis fasciola*, by using eDNA as a function of both biological and technical sampling intensity. This will be accomplished through: mesocosm experiments to yield eDNA shedding rate data and field experiments using caged mussels to determine how eDNA behaves in riverine systems. The

results would have important implications for freshwater mussel conservation and further eDNA methods for use by parties which are required by law to sample for endangered aquatic organisms prior to a new development project.

Can we use eDNA to estimate population sizes? The importance of understanding variation in eDNA production

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Environmental DNA (eDNA) analysis has been demonstrated to be a powerful, non-invasive method for detecting rare aquatic vertebrates. The next frontier for target species eDNA applications is the estimation of population-level information. To accomplish this, we must address the major gap in knowledge regarding the process of production (origin). Differences in individual- or population-level production have been hypothesized to be attributed to variation in physiological state; however, these mechanisms have not yet been directly investigated. To address this research gap, we synthesized the eDNA literature and put findings in the context of environmental physiology, focusing on the effects of life stage, metabolic rate, physiological stress, and disease state on eDNA production, as well as factors affecting post-mortem release rate. A critical pattern that has been observed repeatedly in eDNA experimental studies is that of individuals shedding eDNA at a rate far higher than other individuals in their treatment group (super-shedders). Additionally, several studies have documented individuals shedding orders of magnitude more eDNA at different points in time. This distribution of production rate within and between populations must be accounted for when developing predictive relationships between eDNA copy number and population abundance. We explored potential mechanisms influencing individual variation in eDNA production leading to super-shedding based on known physiological processes and environment-organism interactions. This synthesis of environmental physiology and eDNA literature identifies new avenues of research to forward our ability to gain population-level information from eDNA samples.

Validating eDNA metabarcoding for marine fishes in diverse ecosystems using a public aquarium case study

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Background: Environmental DNA metabarcoding techniques have been regarded as powerful tools for use in the monitoring of biodiversity in aquatic ecosystems. Despite their relatively rapid acceptance in academia and industry, these techniques still require extensive validation due to the existence of several key uncertainties surrounding the use of eDNA metabarcoding. Additionally, these uncertainties are particularly of concern when monitoring highly diverse aquatic ecosystems. In this study, we assess the ability for eDNA metabarcoding to capture biodiversity in a highly diverse closed system at the Ripley's Aquarium of Canada in Toronto, Ontario as well as address key methodological and analytical knowledge gaps pertaining to eDNA metabarcoding to promote a discussion on current issues limiting the application of these techniques.

Results: This study found that eDNA metabarcoding recovered 62 of 107 target species and 30 of 44 target genera from a closed system when using a multi-marker (*COI, 16S, 12S*) approach. Disparity in off-target identification noise was found across these markers with maximum detection only achievable when using all markers. Lastly, issues in species detection were detected regardless of abundance within the system.

Significance: This case study identifies several outstanding issues with eDNA metabarcoding relating to marker selection, data validation and confidence, and the complexity of abundant and diverse novel systems. We conclude that the key uncertainties of eDNA metabarcoding methodology identified here require further focus before eDNA metabarcoding can be broadly applied for aquatic biodiversity monitoring.

Comparative analysis of E-DNA and live-trapping sampling techniques for Jefferson Salamander

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Identifying the presence of species at risk in a project study area is an essential first step in wildlife conservation and management. Jefferson Salamander (*Ambystoma jeffersonianum*) is an amphibian listed as endangered under Ontario's Endangered Species Act 2007 with a historic range that extends into southern and central Ontario. In our poster we compare the benefits and challenges associated with using E-DNA and conventional live-trapping sampling techniques for detecting Jefferson Salamander at a project site in Hamilton, Ontario. Our comparative analysis includes commentary on survey planning, timing, effort and methodology, regulatory agency considerations, and survey results.

The potential use of environmental (e)DNA to detect Chinese mystery snails, *Cipangopaludina chinensis*, an aquatic invasive species in Atlantic Canada

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The Chinese Mystery snail, *Cipangopaludine chinensis*, is an invasive (non-native) mollusc in North America. *C. chinensis* is from the mystery snail family, Viviparidae, and is native to East Asia. Invasive species threaten the biodiversity and species at risk in Canada by out competing native species for available resources. To assess the potential impacts of *C. chinensis* on native species and species at risk, lake surveys must be completed to determine in which lakes *C. chinensis* has invaded. In addition to more traditional survey methods which focus on formal and informal survey methods for freshwater organisms (specifically mussels), environmental DNA (eDNA) is an innovative, low cost method that could detect the presence of invasive species in freshwater ecosystems. Traditional survey methods are more disruptive to the ecosystem, time consuming, and can be expensive. eDNA offers the ability to analyze multiple samples from a variety of lakes, rivers, and ponds, for low costs, it is much less invasive than formal and informal surveys, and can detect multiple species from one sample. We compared two types of survey methods, an informal and a formal random stratified method, and try to create a new eDNA method for future mollusc freshwater surveys.

The relationship between eDNA signal and organism abundance in large mesocosms

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Interest in using eDNA for population and biodiversity monitoring is growing exponentially. Despite this surge in application, knowledge gaps still exist concerning both how eDNA interacts with the aquatic environment and what ecological information can be inferred from its signal. For example, the relationship between eDNA and organism abundance is not fully understood and has been explored in limited spatial and temporal settings. The Limnotron facility, a series of six 26,000 L lentic mesocosms located within the Biodiversity Institute of Ontario, provides a unique opportunity to explore this important aspect of eDNA ecology at larger scales. Four tanks containing populations of *Daphnia magna* were regularly sampled over four months for both organism abundance and the target species' eDNA. Preliminary data suggests a positive correlation between *D. magna* abundance and eDNA signal, but not without effects from DNA persistence and organism decomposition. Estimating organism abundance from eDNA is one of the most desired applications of molecular-based biomonitoring. As such, elucidating the nature of this relationship in controlled environments is paramount for appropriate use of this tool in the field.

A robust eDNA detection method widens the known distribution of the coastal tailed frog in British Columbia in five sampling days compared to four years of time-constrained search activities

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The coastal tailed frog (*Ascaphus truei*; ASTR) is provincially yellow-listed by the Conservation Data Centre and is listed as Special Concern under Schedule 1 of the *Species at Risk Act*. In Canada, coastal tailed frog occurs only in British Columbia where the

species widely occurs west of the Coast Mountain Ranges; with their range extending north almost as far as the Alaskan panhandle. The present study focused on surveying within the Cayoosh, Bridge (Shulaps), Seton, Anderson, Carpenter, and Downton Lake drainages. Four years of previous inventory efforts using conventional time-constrained search (TCS) methods detected tailed frog at 22/288 discrete sites for a detection rate of 7.6% in seven watersheds. A total of 72 sites within the study area were sampled using environmental DNA (eDNA) methods over five days. We rigorously validated a quantitative real-time polymerase chain reaction (qPCR)-based tool for detecting ASTR DNA and applied a two-step targeted eDNA analysis approach on filtered water samples. The first step, designed to mitigate false negative results, tested all DNA samples for the ability to support amplification from endogenous plant chloroplast DNA as a measure of sample viability. Viable DNA samples were then tested for species-specific DNA. ASTR eDNA was detected in 33/72 discrete stream reaches; 10/11 sites with historical known ASTR occurrence were eDNA positive. The results expanded known ASTR distribution to 16 watersheds effectively more than doubling confirmed extant occurrences and confirmed a previously suspected, apparently isolated ASTR metapopulation in the Shulaps drainage.

Quantitative eDNA assessment of reintroduced Atlantic salmon (*Salmo salar*) in Lake Ontario tributaries using microsatellite markers

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Until the late 1800s, Atlantic salmon (*Salmo salar*) were abundant and part of Lake Ontario's native fish community, now extirpated due to human impacts. Plans to reintroduce a population of Atlantic salmon in Lake Ontario consists of stocking hatchery-reared fish yearly which will help to achieve a self-sustaining population. The issue with reintroduction remains in understanding the distribution of fishes after stocking. Thus, it is important to monitor the fate of these fishes, to assess post-release practice and progress. Environmental DNA (eDNA) provides a sensitive approach for monitoring that can offer inferences into fish distribution. Distribution of fish eDNA may not be only useful for determining presence and absence, but also for abundance. Through the use of alternative molecular markers, eDNA can be used to quantify stocked Atlantic salmon in Lake Ontario tributaries to determine their absolute abundance. Methods will include sampling stocked and control unstocked streams. eDNA will be characterized first to identify Atlantic salmon presence using COI markers. The same eDNA will be used to quantify abundance at positive hit sites using microsatellite markers. The resulting PCR products will be NextGen sequenced to determine the number of alleles present in the water at each locus. Allele counting models will be used to estimate the number of individuals contributing to the eDNA signal. This data will help determine which areas reintroduced Atlantic salmon may concentrate in, indicating preference of habitat, as well as provide an estimate of survival. Establishing a self-sustaining population of Atlantic salmon will help restore a native species to Lake Ontario, thus providing a baseline for reintroduction and management of other native species.

ssPRIMER - An open-source, GUI-based software tool for design of species-specific primers and Taqman probes with implications in eDNA research

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Quantitative-PCR (qPCR) is a technique that has been shown to have great promise in the field of environmental bio-monitoring, more specifically in environmental DNA (eDNA) detection. Here we present ssPRIMER (or species-specific PRIMER), an open-source, web-based software tool that can be used to easily design species-specific primer sets and Taqman probes for qPCR assays. A multiple sequence alignment can be imported in by a user, and the tool will then guide the user through the process of designing and evaluating species- specific primer and probe sets with a user-friendly interface. The tool is designed to create primer and probe sets that maximize amplification efficiency for the target species (sensitivity) but minimize amplification efficiency for non-target species (specificity).

Overall, this tool comes with several advantages over pre-existing tools. It will soon be openly accessible on Github with the source code being open to distribution and modification under the GPL (General Public License) license. It uses the web-based platform Shiny, thus avoiding operating system-specific compatibility issues. It provides useful evaluation options for the designed primer sets, including annealing temperature curve analysis and primer and probe binding visualization. Lastly, it offers simplicity in the UI design and provides helpful instructions throughout the design, evaluation, and export process.

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Pathway to Increase Standards and Competency of eDNA Surveys (PISCeS)
A National Workshop on Environmental DNA

This tool is designed to benefit the users of eDNA technology, including field biologists, ecologists, conservation researchers, and environmental consultants and could contribute to environmental bio-monitoring using molecular methods.

Quantifying variation in eDNA for aquatic insect community datasets in streams

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Incorporating environmental DNA (eDNA) into both aquatic community ecology and biological monitoring programs can be advantageous, especially for aquatic insect communities. In addition to overcoming morphological identification constraints (e.g., juvenile or damaged specimens, coarse level identifications), previous research has suggested that eDNA is more sensitive to rare species than traditional kicknet collection for stream macroinvertebrates. However, there are challenges surrounding the use of eDNA in field assessments as uncertainty exists surrounding the fate and transport of genetic material in aquatic systems. In streams, eDNA is expected to flow downstream and it is unclear how this influences variation in samples collected at the same location. The uncertainty surrounding the fate and variation of eDNA in riverine systems thus requires more detailed methodological studies before eDNA can be reliably used in place of kicknet collection, as large variation in community datasets at small spatial scales could bias conclusions.

To address these methodological concerns, we have collected both eDNA and kicknet samples in nine headwater streams in the Lake Erie watershed in order to quantify the taxonomic variation present in each method. At each sample point, we have collected 3 eDNA samples and 3 kicknet samples to 1) assess the variation in community composition within each sampling method at the same location, 2) assess the variation in community composition between methods and 3) observe whether the aquatic insect community datasets generated by each method respond to the same environmental variables (e.g., water chemistry, land use).

eDNA in the Field: Identifying the Factors that Influence the Detection of Benthic Macroinvertebrates using Environmental DNA in the Subarctic

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During August 2018 we sampled caddisflies (Trichoptera) in Churchill, Manitoba in the context of a multi-year study on their phylogeny and habitat choice. Caddisflies are widely used as bioindicators due to their sensitivity to pollution and therefore would benefit from a more rapid sampling method, such as using environmental DNA (eDNA). Due to the historical data we have on their distribution around Churchill, this system is ideal to validate eDNA-based assays for these benthic macroinvertebrates in a novel environment. We targeted a particularly widespread and abundant species (*Philarctus bergrothi*) for this study and sampled 24 coastal ponds for their presence using both eDNA and traditional sampling methods (i.e. kicknetting and rock washing). Species-specific primer and probe sets were designed, and targeted qPCR tests were run on the extracted eDNA sample from each pond. Using a portable and minimal lab setup, we were able to obtain presence/absence data at a field station shortly after sampling. By comparing the proportion of positive qPCR replicates between samples and considering the variation in water chemistry and abundance of target organisms between ponds, we can determine which factors most affect the detection of macroinvertebrates using eDNA in subarctic coastal ponds. The successful development of standardized, validated eDNA assays will enhance our ability to survey Arctic biodiversity, detect invasive species, and track shifts in distribution caused by climate change.

Combating sea lice in Canadian aquaculture by utilizing targeted qPCR and metabarcoding to determine cleaner fish diet

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Sea lice (*Lepeophtheirus salmonis*) pose a substantial concern for aquaculture. Canadian salmon aquaculture relies on the Lumpfish (*Cyclopterus lumpus*) and the Cunner (*Tautogolabrus adspersus*) as biocontrol species to remove sea lice. A large variation in lice-removal efficiency exists between and within the two species, so detailed information regarding diet preferences must be collected. However, since both species possess generalist diets containing hard to identify taxa, it is difficult to assess their trophic ecology using traditional methods. Furthermore, the ability to collect repeated measurements on the same individual is complex due to ethical

concerns using methods like gastric lavage. The aim of this study is (i) to assess differences in diet between wild and caged lumpfish and cunners, and (ii) to study the application of targeted amplification of lice eDNA extracted from cleaner fish stomach liquid to assess cleaner efficacy. The following methods will be used: targeted qPCR for sea lice in eDNA extracted from stomach liquid aliquots, morphological examination of stomach contents, and metabarcoding of DNA extracted from stomach contents. This will help improve the sustainability and profitability of Canadian Aquaculture.

eDNA Solutions for Species Detections in Tidal Energy Environmental Effects Monitoring

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Conducting environmental effects monitoring (EEM) investigations of tidal in-stream energy conversion (TISEC) devices in marine ecosystems is challenging in a naturally variable environments, particularly macrotidal systems like the inner Bay of Fundy (iBoF). Our project applied a novel technology for *in-situ* rapid species identification in high flow marine conditions using environmental deoxyribonucleic acid (eDNA) tools to build upon scientific evidence gained from national and international tidal turbine EEM programs. As eDNA is sampled from non-living ecosystem components, this new technology provides an entirely non-invasive means of conducting ecological surveys without physically harming organisms, particularly species-at-risk, and at a considerably lower sampling effort and cost. Multiple related experiments in replicate tower tanks with varying densities of striped bass (*Morone saxatilis*) were established at Dalhousie University's Aquatron over a six-week period to: (a) assess eDNA detection efficiency and signal persistence; and (b) quantify striped bass density based on the eDNA prevalence. This presentation will discuss preliminary study results and methods to overcome gaps in data collection and interpretation using traditional monitoring tools to assess marine fish populations.