ENVIRONMENTAL DNA: IMPLEMENTATION FOR RESOURCE DEVELOPMENT PROJECTS IN BC AND BEYOND

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ABSTRACT

Effective environmental protection, stewardship, and restoration require timely and accurate information about the status of a given ecosystem and the species that occupy it. Animals shed DNA (deoxyribonucleic acid) as they complete their life processes, and this environmental DNA (eDNA) can be detected via analysis of samples collected from occupied habitats. Studies of eDNA have gained scientific and regulatory acceptance especially for the survey of at-risk aquatic and semi-aquatic species. The field effort associated with sample collection is less than that associated with traditional baited trapping, electro-shocking and/or physical searches, thus enabling more efficient data acquisition over space and time. In addition, this method is non-invasive to the target species and its habitat, reduces the risk of pathogen transfer between sites, is highly accurate, is very sensitive to detection of aquatic species, is able to detect the presence of pathogens and generally is more cost-effective for species that are difficult to detect using traditional methods. The credibility of eDNA survey data, however, depends on adequate methodological validation and verification; accurate results require rigour during field sampling, sample processing, laboratory analysis and primer design and/or verification. Hemmera recently developed accepted standards for collection of eDNA for the BC Ministry of Environment. The completion of more than 20 eDNA projects in British Columbia and the Yukon since 2014; for 18 aquatic taxa, including fish, amphibians, water shrews and pathogens, has provided clear evidence of the utility of this approach. This paper discusses the strengths and limitations of eDNA as a tool for addressing baseline data and monitoring requirements and assessing the effectiveness of reclamation efforts.

KEY WORDS

environmental DNA, eDNA, species at-risk, aquatic, validation, standards

INTRODUCTION

There are significant challenges with managing mining approvals, operations, closure and reclamation, in a manner that results in acceptably low environmental impacts. These challenges are a consequence of the large spatial and temporal scale of disturbance for a typical mine project, in concert with the fact that most mines are located in undeveloped functional wildlife habitat. Inadequate information regarding the ecological distributions of biological species of management interest can severely hamper management

decisions, and undermine management outcomes. For example, uncertainty regarding the spatial distribution of at-risk species can lead to conflicting opinions about proposed landscape- and watershed-scale modifications and mitigation. Out-competition by invasive species in an area being reclaimed can undermine ecological restoration goals if the invasive species are not detected early and managed appropriately. Limitations in the ability to track recruitment of valued species to habitat created during mine reclamation will also limit the ability to adapt and maximize the achievement of the stated goals. This paper discusses the role of environmental DNA (eDNA) surveys for describing the spatial and temporal distributions of species of management interest, with a focus on aquatic and semi-aquatic at-risk or otherwise valued aquatic taxa. Adoption of eDNA approaches in mining-related environmental decision making is motivated by the fact that eDNA has the potential to substantially improve our ability to pragmatically and cost-effectively gather relevant data when compared with traditional survey methods, and substantially improved knowledge about the spatio-temporal distribution of species of management interest will generally improve the ability to balance environmental protection, effective and efficient mitigation and mining economic goals. The ease with which data can be obtained allows more efficient and accurate spatial and temporal resolution of species' distribution and is inversely proportional to the magnitude of uncertainties about ecosystem state and functions.

BACKGROUND

Living organisms routinely shed, into their surrounding environment, dead skin cells, gametes, and various other tissues that contain their genetic material (deoxyribonucleic acid, or DNA). For aquatic and semi-aquatic species, this exogenous environmental DNA (eDNA) can be collected through filtration of water samples, preserved, and subsequently assayed in an appropriately equipped and experienced laboratory to detect the presence of DNA from aquatic and semi-aquatic species (Fig. 1). This method is referred to as eDNA and provides a promising and emerging method for more cost-effective, less invasive and more efficient survey of aquatic species living in both lotic and lentic systems (Biggs et al. 2014; Herder et al. 2014).

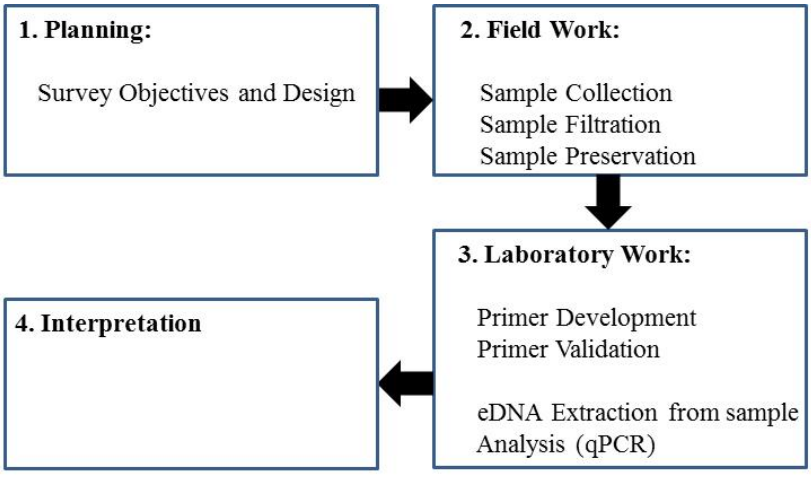

Figure 1: Sequence of steps in eDNA method

Environmental DNA methods are currently being used to survey a diverse suite of freshwater environments including lotic (e.g., stream) and lentic (e.g., wetland) systems for a variety of aquatic and semi-aquatic species including benthic invertebrates, fishes, amphibians and semi-aquatic mammals (Goldberg et al. 2011, Thomsen et al. 2012, Farrington and Lance 2014). The reliable detection of aquatic vertebrate species using eDNA, from a variety of freshwater systems, has been confirmed as an effective survey method for many species (Ficetola et al. 2008, Goldberg et al. 2011, Jerde et al. 2011, Thomsen et al. 2012). eDNA can also be recovered from other environmental matrices such as soils or sediment, and - particularly in anoxic environments – this may confer increased environmental persistence of genetic material as a direct result of reduced biodegradation and photolysis rates (Turner et al 2015). There are, however, additional theoretical and methodological challenges associated with eDNA surveys using soil or sediment deposits as application of eDNA practices to sediment sampling are just evolving. By contrast, eDNA practices for surface water sampling are quickly becoming well established and accepted in the literature and in operational practice.

Water (and/or sediment) samples from environments potentially inhabited by the species in question are collected and field preserved for subsequent analysis in the laboratory to detect the presence of DNA for the target taxa. Laboratory analysis is generally carried out using quantitative polymerase chain reaction (qPCR) methods as described in Veldhoen et al (submitted 2016). The PCR process enzymatically increasing the abundance of a specific sequence of DNA from target taxa in a sample by repeatedly subjecting the sample to alternating heat (to denature, or separate, the double helix) then cooling to copy the original DNA. This process is repeated many times (e.g. for 50 cycles) resulting in an exponential increase in copy number of the DNA from the target taxa. Finally, amplicons are detected using a species-specific probe. Each process, including all cycles, is referred to as a run and each environmental sample collected is typically run between three to eight times for each sample collected, to arrive at a binary conclusion regarding presence or absence of DNA from the target taxa. Samples from each site are then considered collectively to derive support for a positive or negative assignation (for the presence of DNA from the target taxa) at the site level. The use of a qPCR assay requires development of species-specific assays (primers and probe) that target a small section of the genome: both primer specificity and sensitivity are key to this process (Farrington and Lance 2014).

As eDNA methods are refined and further validated, the approach is becoming an increasingly widespread tool for biological assessment of species distribution in aquatic systems, particularly for inventory of at-risk and invasive species (Jerde et al. 2011, Goldberg et al. 2015). Survey methods based on eDNA are attractive when considering limitations of more traditional survey approaches. In particular, traditional survey techniques for rare or elusive species are often labour-intensive and inefficient, yield much lower relative detection probabilities, are invasive to the target species, often deleterious to the species' habitat, and require involvement of experienced professional biologists. In contrast, eDNA surveys offer greater sensitivity for species detection, reduced field time and expense, and are non-invasive to the target species and habitat. In addition, field sampling can be conducted by field personnel with minimal training and with less restrictive conditions for appropriate survey timing (e.g., eDNA can be conducted outside amphibian calling windows) and under a wider range of environmental conditions (e.g., extreme winter conditions when species are dormant and typically undetectable using conventional methods).

Invasive species present a similar opportunity for the application of eDNA methods. The most opportune time to prevent the population growth, or spread, of an invasive species is when it is first introduced, and is present at low density. By virtue of its sensitivity, eDNA technology is most useful when the target species is present in low density, precisely the scenario where traditional survey methods are least effective. By using eDNA techniques, managers can detect invasive species early, and employ mitigation resources more cost-effectively.

A more detailed overview of the advantages and limitations of the eDNA method, based on the best available information from the literature, is provided by Herder et al (2014). Hobbs and Goldberg (2016) details application of eDNA in a provincially and federally relevant management context and provides a standardized and government accepted protocol for eDNA studies in British Columbia (BC) for both lotic and lentic freshwater ecosystems. The protocol discusses recommended approaches for study design, outlines the steps required for tissue and surface water sample collection, and provides guidance for reporting and discussion of results.

Environmental Persistence of Exogenous DNA

There are many published paleo-ecological studies of long term chronologies that use genomic approaches (amplification and identification of "fossil DNA") for the examination of ecological, hydrological and climatic shifts in areas around depositional basins (Boere et al 2011). In contrast, there is a limited persistence over time of exogenous DNA in aquatic surface-water samples that are the focus of eDNA studies. This is because of the relatively low expected aqueous concentrations at the time of introduction by the species of management interest (potentially resulting in false negatives) and reduced specificity of primers that recognize more degraded fragments of eDNA (potentially resulting in false positives).

Understanding the rate of eDNA degradation for the target organism in the aquatic system is informative when determining extant occurrence of target taxa. The published rates of eDNA degradation from controlled laboratory experiments vary depending on local conditions. A consistent pattern that emerges, however, is an exponential decay of detectable eDNA over time (Thomsen et al. 2012, Barnes et al. 2014, Strickler et al. 2015), with persistence half-lives ranging from than one hour in a lotic system (Pilliod et al. 2014) up to approximately 58 days under controlled laboratory conditions (Strickler et al. 2015), with 7 to 25 days being the mid-range (Dejean et al. 2011, Thomsen et al. 2012).

Temporal persistence of eDNA in natural systems depends on several factors including: water temperature, flow rates in the system (e.g., lotic or lentic), level of exposure to ultraviolet rays, pH, salinity, substrate type and microbial community activity (Strickler et al. 2015). The duration of persistence of eDNA in a system is influenced by local environmental conditions at the time of sampling (Strickler et al. 2015). While no general conclusions for eDNA persistence rates can yet be drawn across taxa or systems, a conservative guideline for persistence of eDNA in an aquatic system is 7 to 21 days after the removal of the organism from the system.

At least one study (Turner et al. 2015) has shown that eDNA persistence half-lives may be greater in sediments than in water.

Comparison of eDNA surveys with Traditional Approaches

eDNA becomes an increasingly favourable and cost effective alternative for the evaluation of spatiotemporal distributions of inconspicuous species that are characterized by low population densities and/or discontinuous distributions (Figure 2). In cases where a species is highly detectable based on visual or audible cues, conventional methods may be preferable to eDNA.

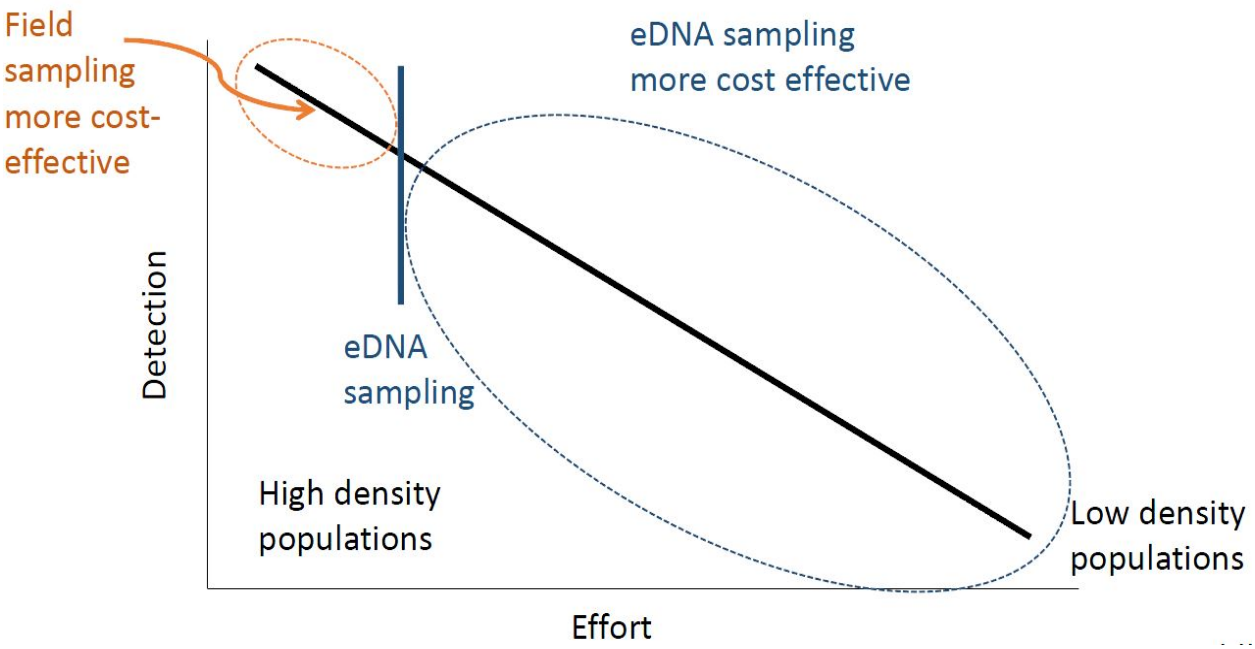

Figure 2: Comparative utility of traditional survey techniques (i.e. field sampling) versus eDNA

The most obvious benefit of eDNA is increased efficacy (i.e. detection probability) (Table 1). This is a result of sensitivity of qPCR techniques and the associated ability to detect exogenous DNA from target taxa in trace quantities. By comparison, conventional methods typically demonstrate greatly reduced efficacy in relation to the degree of skill held by individual observers, potential observer bias, and challenges in conducting visual, auditory or other search methods within potentially viable occupied habitats, particularly for evasive species or species with inconspicuous life histories. To achieve a parallel level of efficacy with eDNA, conventional methods would typically require a much higher degree of replication across space and time.

Conventional methods are also heavily influenced by observer skill. The retention of adequately skilled observers for any inventory program will generally increase the associated cost. Conversely, eDNA sample collection can easily be completed, without observer (skill) bias, by technicians with very little formal training or experience with the specific target taxa. Additional insights and efficiencies can be gained, nonetheless, through the engagement of surveyors with expertise in both the species of

management interest and familiarity with conventional observation-based methods when combined with eDNA. To increase cost-effectiveness prudent study design may incorporate an adaptive (hierarchical) decision structure that stipulates that eDNA methods will only be applied if the species is not incidentally detected at a site during sample collection or brief site assessment. Furthermore, a combination of existing information from prior survey based on conventional methods and/or incidental observations during sample collection, incorporated within an eDNA survey program with much larger spatial or temporal scope, provides additional validation of eDNA study results with observation based confirmation of positive qPCR results.

Table 1: Systematic comparison of practical issues associated with conventional versus eDNA methods

ATTRIBUTE	CONVENTIONAL METHODS	eDNA
Efficacy (detection probability if species present)	Low-High	High
Multi-species survey potential	Sometimes	Yes
Post-sampling/observation evaluation of additional taxa	No	Yes
Adaptive design and testing	No	Yes
Vulnerability to observer bias	High	Low
Permitting required	Yes	No
Invasiveness to species and critical habitat	High	Low
Pathogen transfer risk	High	Low
Window of opportunity for field surveys	Highly constrained	Less constrained
Special needs including expert knowledge, training, equipment	High	Minimal
Safety considerations	Medium-high	Low

Potential Limitations of eDNA

There remain several outstanding issues with regard to eDNA methods and their interpretation. The ability to detect and eventually quantify local population abundance (or organismal densities) for a semi-confined aquatic ecosystem depends on the influence of three major intrinsic factors:

- Environmental input which includes organismal shedding rates of exogenous DNA and distance to source of genetic material from the target taxa;
- Environment re-distribution and compartmentalization; and
- System attributes that influence eDNA degradation rates.

In addition, extrinsic factors that potentially influence accuracy of results in eDNA applications include methodological uncertainty and variation, including false negatives and positives, associated with sample capture, preparation, preservation, and analysis (Goldberg et al., 2015).

Variations in eDNA field sampling/processing/preservation methods and laboratory based PCR or qPCR approaches can lead to both false positives (especially for the high degree of amplification inherent in

detecting small masses of eDNA, based on imperfect primer specificity, or based on sample cross-contamination) and false negatives (e.g., based on loss of DNA during sampling and preservation or based on inhibition) (Ficetola et al. 2015; Goldberg et al. 2015; Thompsen & Willerslev 2015). The ability to derive meaningful species presence or abundance data from analysis of smaller fragments of mitochondrial DNA may be more limited for degraded eDNA in particular.

MINING-RELATED APPLICATIONS

The ability to accurately survey and monitor biological species distributions may be important for the following:

- Evaluation and mitigation of possible impacts of proposed mining projects and expansions to at-risk and otherwise valued vertebrate (mammalian, avian, reptilian and amphibian) wildlife, fish, invertebrates, and plants (for example, as required for mining project environmental assessments);
- Effective management of mitigation strategies involving salvage and relocation;
- Clarification of the distributions of species selected for environmental effects monitoring;
- Tracking and managing against introduced species, including potentially important wildlife pathogens, during mine operations and during reclamation; and
- Evaluation of the success of reclamation efforts to support habitat creation for desired species, based on range extensions in newly developed habitat.

The limited aqueous environmental persistence of eDNA, as discussed previously, can be helpful for mining-related applications. In particular, the limited persistence in water of eDNA for most species of management interest may allow determination of seasonal distribution of target taxa, as well as synoptic increases or decreases in distribution over time.

CASE EXAMPLES

World-wide, eDNA studies have proliferated since 2008, with more than 100 peer-reviewed studies published to date. There has been a rapid increase in the number of studies annually each year over the last half decade. A small example of taxa that have been evaluated based on published eDNA studies, and including studies completed in BC by Hemmera, include the following:

- Pathogens (including Ranavirus and *Batrachochytrium dendrobatidis* (Bd)) (Taxa assessed, using eDNA, by J.Hobbs)
- Invertebrates: trematode pathogen of amphibians (Laramie et al. 2015) and dragonflies
- Fish: invasive and native carp species (Klymus et al. 2015); European weather loach (Sigsgaard et al. 2015); fifteen marine fish species (Thomsen et al. 2012); Taxa assessed, using eDNA, by J.Hobbs include Arctic Grayling (*Thymallus arcticus*), Chinook (*Oncorhynchus tshawytscha*), Bull Trout (*Salvelinus confluentus*) (Hemmera 2015)
- Amphibians: great crested newt *Triturus cristatus* (Biggs et al. 2015, Rees et al. 2014); hellbender *Cryptobranchus alleganiensis* (Spear et al. 2015)]; Taxa assessed, using eDNA, by J.Hobbs include coastal giant salamander (*Dicamptodon tenubrosus)*, coastal tailed frog

(*Ascaphus truei*), rocky mountain tailed frog (*Ascaphus montanus*) , red legged frog (*Rana aurora*), Oregon spotted frog (*Rana pretiosa*), leopard frog (*Lithobates pipiens*), Columbian spotted frog (*Rana luteiventris*), Cascades frog (*Rana cascadae*), western toad (*Anaxyrus boreas*), tiger salamander (*Ambystoma mavortium*), American bullfrog (*Lithobates catesbeianus*), Great Basin spadefoot (*Spea intermontanus*)

- Reptiles: eight turtle species native to Canada (Davey et al. 2015); invasive Burmese pythons (Hunter et al. 2015)
- Mammals: Taxa assessed, using eDNA, by J.Hobbs include Pacific water shrew (Hemmera 2016)
- Plants: invasive aquatic plants (Scriver et al. 2015)

eDNA studies completed or in progress, by J. Hobbs, since 2014 in British Columbia and the Yukon are listed in Table 2.

The use of eDNA surveys to develop a better understanding of the spatial distributions of at-risk species that might otherwise be impacted by mine developments is an intuitively obvious application of this new but proven technology. Depending on rates of shedding of DNA into surface waters, and depending on rates of mixing and dilution of the water-borne DNA signal by contributions from runoff in the surrounding unoccupied watershed, eDNA surveys within watersheds can be used diagnostically to rapidly assess candidate sub-watersheds that may support a species of management interest, and in turn to provide focus to increased efforts within on extant habitats. This can be helpful to more accurately predict the proportion of a local population that could be affected by a development, or to identify suitable habitat for relocations and population recovery efforts.

Many of the environmental issues associated with mining consider fisheries and aquatic habitat. A recent application of eDNA involved the critical evaluation of the seasonal arctic grayling (*Thymallus arcticus*) distribution in several small tributaries of the Yukon River. In particular, there was an interest in confirming the existing conceptual understanding from traditional fish and fish habitat surveys of stream use by grayling. Especially in more mountainous regions that host mineral deposits of economic value, use of stream habitat by fish may change appreciably from lower to higher elevation reaches, and there is significant variation in fish presence within a specific reach during open-water summer-time periods versus in the winter under ice. The reaches of interest were sampled in the winter-time through the sampling of both flowing water and streambed sediment via auger holes through up to 1.3m thick ice cover. The sampling occurred sufficiently long after freeze-up that it was highly unlikely that exogenous DNA in flowing water would be present as a result of historical (summer) fish presence and shedding during open flow (summer) conditions. Results from this study served to confirm extant use at several known locations and served to identify several new areas of overwintering grayling habitat that had not been confirmed despite prior application of conventional methods.

CONCLUSIONS

The simplicity of sample collection and the sensitivity of eDNA survey and analysis using qPCR makes

Table 2: Specific examples of British Columbia and Yukon eDNA Projects by J. Hobbs since 2014

APPLICATION	SPECIES	AREA	YEAR
Highway improvement project (1)	red-legged frog/Pacific water shrew	BC Lower Mainland	2014
Highway improvement project (2)	red-legged frog/Pacific water shrew	BC Lower Mainland	2014
Conservation management (MFLNRO)	tiger salamander/spadefoot toad	BC Okanagan	2014
Conservation management (MFLNRO)	rocky mountain tailed frog	BC Flathead	2014
Conservation management (MFLNRO)	red-legged frog/Pacific water shrew	BC Lower Mainland	2014
Conservation management (MFLNRO)	coastal giant salamander	BC Lower Mainland	2014
Conservation management (MFLNRO)	Pacific water shrew & red-legged frog	BC Sunshine Coast	2014
Highway improvement project (1)	red-legged frog/Pacific water shrew	BC Lower Mainland	2015
Highway improvement project (2)	red-legged frog/Pacific water shrew	BC Lower Mainland	2015
Port of Vancouver species at risk inventory	red-legged frog/Pacific water shrew	BC Lower Mainland	2015
Screening environmental assessment	red-legged frog/Pacific water shrew	BC Lower Mainland	2015
Conservation management (MFLNRO)	Rocky Mountain tailed frog	BC Wigwam	2015
Conservation management (Yukon CDC)	western toad/Columbia spotted frog	YT Watson Lake	2015
Conservation management (Yukon CDC)	Ranavirus/BD	YT Watson Lake	2015
Conservation management (Yukon CDC)	Chinook/bull trout	YT Teslin/Kusawa	2015
Mine environmental assessment	Arctic grayling	Central YT	2016
LNG baseline assessment	Red-legged frog/western toad	Southern BC	2016
Conservation management (MFLNRO)	coastal tailed frog	BC Lillooett	2016
Conservation management (Yukon CDC) training	Ranavirus/Bd	YT	2016
Conservation management (HCTF)	Red-legged frog, Oregon spotted frog, Cascades frog, Columbian spotted frog, western toad	BC Lower mainland	2016

the technique an appealing option for application to a variety of mining related environmental questions and issues. While eDNA methods are relatively new, there are abundant examples of the pragmatic use of eDNA methods for better defining the distribution of rare or elusive species. Within the last three to five years, there has been a marked shift in the community of interest: eDNA survey and analytical methods were initially advanced predominantly by academic researchers, with a subsequent completion of specific studies through the support of conservation biologists within the public sector. More recently, however, a far greater percentage of studies have been catalyzed as a result of environmental management needs associated with government led conservation initiatives and industry led infrastructure and resource development (Table 2).

The use of eDNA surveys in British Columbia, the Yukon, and other jurisdictions already garners excellent regulatory support. It is important to acknowledge, however, that some managers in some jurisdictions remain skeptical about the ability of eDNA surveys to produce accurate results. This generally has been caused by prior experience with specific eDNA research studies that lacked adequate field and laboratory methodological rigour and understanding to adequately control false positives or false negatives. For example, eDNA in water may be very labile and is prone to rapid degradation when exposed to sunlight or at higher temperatures. Several studies have failed to account for loss of DNA from sampled media as a result of sampling error. eDNA studies are also particularly prone to sample cross contamination during field sampling as the sampler moves from one candidate habitat area to another as a result of poor collection procedures.

The development within British Columbia of a standardized and government accepted eDNA Protocol for Freshwater Aquatic Ecosystems (Hobbs and Goldberg 2016) should provide greater confidence that results produced using eDNA studies accurately reflect species' spatiotemporal distributions. The United States Geological Survey (Laramie et al 2015) has similarly published parallel sampling and analytical guidance for use in the USA. Just as there are important pitfalls to avoid during field sampling, it is important that the samples be processed and analyzed in an appropriately experienced laboratory, with adequate knowledge about the specific and complex quality assurance and quality management issues that are associated with primer development, sample processing, qPCR amplification and other analytical steps.

Nonetheless, the potential pitfalls associated with eDNA surveys are widely accepted to be adequately understood and easily avoided. Furthermore, traditional survey approaches also contain a variety of pitfalls, perhaps the most important of which is low efficacy and high variability across observers. Above all, eDNA offers promise to pragmatically and cost-effectively increase available and accurate information regarding the spatial density and geographic extent of species distributions. Improved information and understanding of species' distribution, within a geographic area of interest, will better inform decisions regarding resource extraction and reclamation to accommodate ecological conservation and management goals.

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