Invisible Trace Evidence:
Using Environmental DNA (eDNA) to Detect Species in Aquatic Ecosystems

1. Assessing habitat of a larger stream in preparation for eDNA

THE CHALLENGE

Increasingly, priorities for environmental planning and management of transportation projects focus on protection of biodiversity. In Canada, this focus has been reinforced by the provisions of the federal *Species at Risk Act* and complementary provincial legislation. Further, management of invasive species to protect biodiversity, resources, and agriculture often necessitates rapid response, particularly for aquatic invasive species.

Linear transportation projects typically cross multiple watercourses, which can alter adjacent habitat through loss, disruption and fragmentation, and change surface water quality, storage and transport. Understanding potential effects across multiple drainages is of key interest to regulators and is often very costly to the transportation agency.

In recent years, heightened awareness regarding highway infrastructure operating as potential pathways for invasive species has resulted in inter-provincial collaboration to prevent the spread of aquatic invasive species such as zebra and quagga mussels. Such invasives can significantly disrupt local native food webs and reduce populations of species that are of cultural, recreational, and commercial importance.

Hemmera's submission discusses how environmental DNA (eDNA) can be used to quickly and cost-effectively detect aquatic and semi-aquatic species, particularly for at-risk and invasive species.

2. eDNA collection from a smaller stream

THE PRACTICAL NEED

Public and regulatory support for transportation projects can be enhanced where transportation agencies can demonstrate pro-active measures to protect biodiversity and prevent the spread of invasive species. Furthermore, transportation projects can create environmental net benefits when there is a better understanding of species ecology on which to base habitat enhancements. The ability to mitigate and offset risks to important species and habitats is in turn founded on an ability to detect and monitor various species of management interest.

Obtaining baseline or routine monitoring data for at-risk species of amphibians, fish, and other aquatic or semi-aquatic taxa has historically relied on field observations and survey programs by expert biologists, using various specialized traditional ecological survey methods. While these methods are widely accepted, they are generally labour-intensive and expensive to execute in the field, and insufficient funding typically affects the ability to detect and map rare or invasive species occurrences with acceptable rigour.

Traditional survey techniques can be directly detrimental to the viability of rare species due to requirements for:

- intrusive techniques and/or degree of physical habitat disturbance
- handling of target species which may inadvertently transfer pathogens, further threatening the population viability of (endangered) species.

3. Assessing pond (lentic) habitat in preparation for eDNA

THE INNOVATION

Uptake of eDNA applications in transportation-related environmental management is fostered when decision-makers understand how eDNA methods can cost-effectively gather reliable information about the spatial and temporal distribution of species of management interest. Rather than relying on physical capture or sightings of the target species, lab analysis of water samples containing traces of the species' DNA can detect species' presence within a watershed or sub-basin.

eDNA is simply the genetic material (deoxyribonucleic acid, or DNA) of a species that can be isolated from the environment – from surface water samples or other environmental media – and analyzed to infer the recent presence of the species in the sampling area. As we have learned from TV crime scene investigations, living organisms routinely shed dead skin cells, gametes, and various other tissues into their surrounding environment, along with its associated genetic material. This genetic material is detected using quantitative polymerase chain reaction (qPCR) genetic analysis techniques, from which a positive result may indicate species presence. For aquatic and semi-aquatic species, 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 one or more species of management interest.

Use of eDNA was first demonstrated in 2003 where genetic material shed from animals and plants was detected in the environment (Willerslev et al. 2003). In 2008, Ficotela et al. showed that eDNA in pond water could accurately detect occurrences of the highly invasive American bullfrog. Since then, refined approaches for detecting rare and invasive species have ignited interest in eDNA ecological surveys, and its use has grown exponentially. Currently, an average of 30 to 40 new scientific papers on eDNA are published in peer-reviewed journals each month.

Hemmera was the first Canadian environmental consulting company to use eDNA techniques for assessing the presence and distribution of aquatic and semi-aquatic species. While there has been an immense upsurge in use of eDNA in university research, in 2014 Hemmera's practical application in consulting was unique in Canada and elsewhere. Today, Hemmera continues to reflect a best-in-class approach. Hemmera scientists have collaborated with researchers at the University of Victoria and Washington State University on more than 45 eDNA projects in western Canada, examining 18 aquatic taxa, including fish, amphibians, an endangered reptile, semi-aquatic mammals (e.g., Pacific water shrew), and wildlife pathogens. Select projects in southern BC are shown in **Figure 1**.

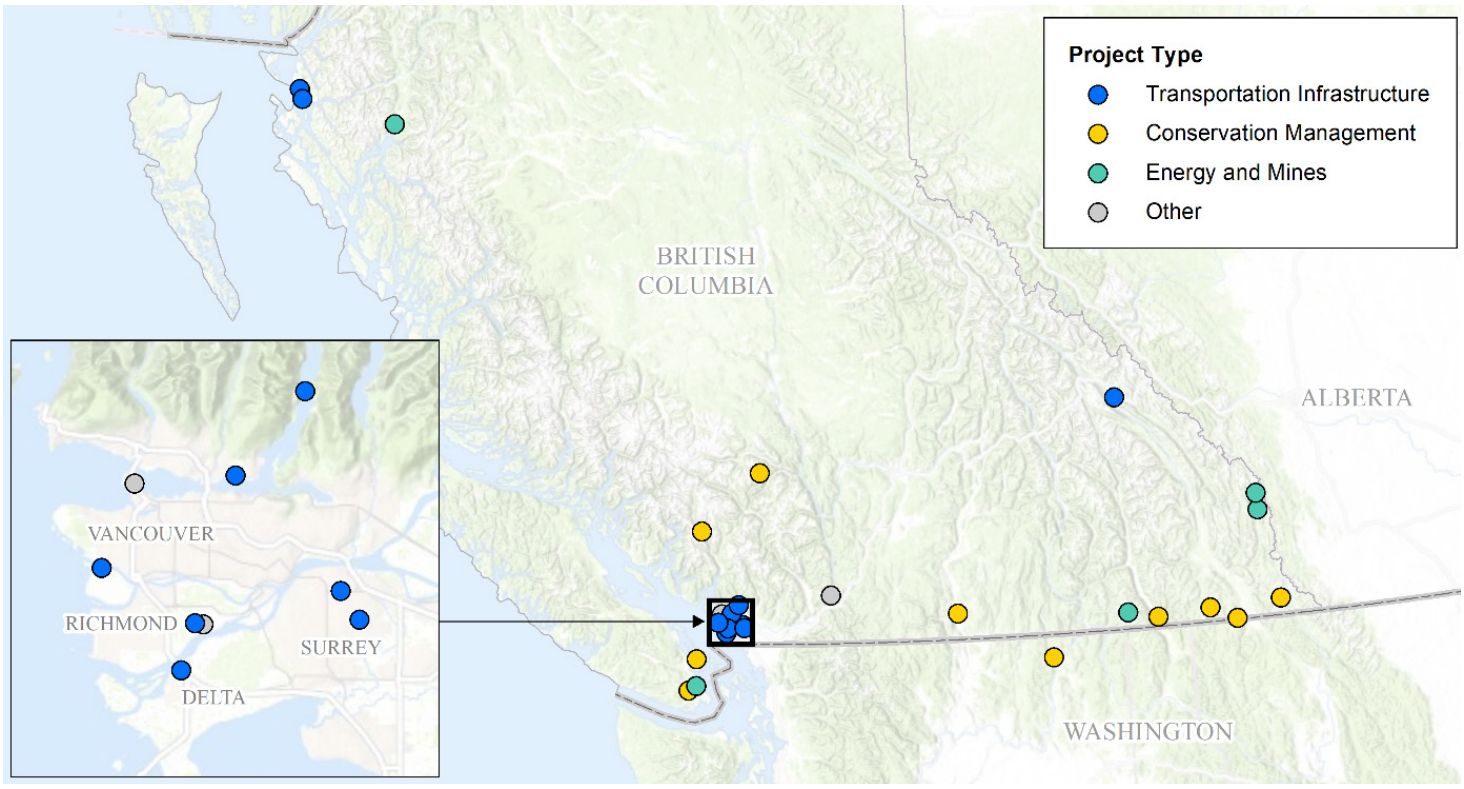

Figure 1 ***Hemmera projects using eDNA in southern British Columbia***

We are currently conducting research on eDNA primers for detection of:

- endangered and invasive freshwater molluscs
- birds that nest and forage near surface water bodies
- bat species that may shed DNA into the water bodies near roosting areas and/or hibernacula
- invasive aquatic plants.

4. eDNA collection from a pond (lentic) system

Hemmera's success in eDNA sampling and analysis provides clear evidence of the utility of this approach. Hemmera has used eDNA techniques to substantially improve delivery of cost-effective, relevant, and accurate data, particularly when compared with traditional survey methods. The spatial and temporal accuracy that eDNA detections of species of management interest has dramatically improved our ability to advise clients on balancing environmental protection and economic development objectives.

A key aspect of the innovation is the extreme sensitivity of eDNA methods for detecting the current or recent presence of a species based on the efficiency of amplifying the DNA "footprint" (qPCR). This approach allows detection of a species even when densities of individuals are extremely low (i.e., rare and endangered species or invasive species recently introduced to a system). In both of these situations, reliable and timely detection may be critically important from a management perspective.

In addition, eDNA survey techniques include:

- describing the habitat (see Photo 1 and Photo 3)
- collecting water samples (see Photo 2 and Photo 4)
- filtration of samples (see Photo 5, Photo 6, and Photo 7).

When compared to traditional ecological surveys, eDNA requires minimal field effort, and is relatively inexpensive to implement, allowing a "bigger bang for the buck" (both spatial and temporal). Thus, adoption of eDNA survey methods is well aligned with the "big data" approaches and decisions that are needed to assist with planning and mitigation, with significant implications for more effective environmental management at larger scales than previously feasible. These eDNA approaches support the increasing interest in, and reliance on, the assessment and monitoring of environmental effects.

We have used eDNA to:

- verify presence/absence of species as part of baseline assessments
- detect multiple species with a single field sampling event (rather than multiple specialized surveys using in traditional sampling)
- monitor colonization of newly constructed habitat by a species of interest
- assess efficacy of invasive species control programs
- expand the known distribution of rare species (e.g., for identifying overwintering habitat for arctic grayling when traditional fish census approaches proved impracticable).

Pacific Water Shrew © Peter Preece, wildlife photographer

CASE STUDY (PACIFIC WATER SHREW)

Prior to advent of eDNA use in 2014, for baseline assessments on major transportation projects in Metro Vancouver (e.g., Port Mann/Highway 1 Improvement Project, South Fraser Perimeter Road), provincial standards dictated intrusive requirements for determining presence of and salvaging for Pacific water shrew (PWS), a species on Schedule 1 of the federal *Species at Risk Act*. Salvage entailed 8 days of continuous monitoring of pitfall traps within segregated sampling areas. Segregation of habitat typically required silt fencing to be dug in around the targeted watercourse, resulting in habitat disruption. Checking of traps had to occur at set intervals 24 hours per day and meant that biologists were in the field at all hours, which was very costly and potentially a worker health and safety issue. Despite sampling in strict observance of the protocol, mortalities of this endangered species did occur. Salvage costs in accordance with the protocol ranged from $20,000 to $40,000 per hectare, depending on the contractor and the size of the area that required salvage. As noted by one of our senior wildlife biologists: "For SFPR and other projects when eDNA when unavailable, we had to salvage widely, at great expense because we could not prove absence of PWS."

By comparison, eDNA sampling costs (including staff and lab time) have averaged $4,000 to $5,000 per hectare. Overall, eDNA costs (to confirm presence/absence of a species) range from 12% to 25% of the cost of a salvage operation. In addition to the cost savings, sampling using eDNA protocols, can be completed:

- in one water sampling event
- with analysis completed at a fraction of the conventional sampling costs
- safely during daylight hours
- with no disruption of habitat
- with no sampling mortality.

Use of eDNA has helped prove PWS absence and avoided the need for high cost salvage operations. We note that eDNA is not a replacement for salvage – rather eDNA is a relatively economical, non-intrusive and efficient means of determining whether salvage is required.

5. Storage to preserve eDNA samples prior to filtration

IMPORTANT CONSIDERATIONS

Key considerations in development of eDNA sampling programs are understanding that:

- eDNA degrades over time at different rates depending on the habitat and exposure to light and oxygen
- water velocities and mixing rates affect sampling design
- there are now standardized field sampling methods
- primers for reliable detection of target species are of variable quality
- skepticism in efficacy of eDNA.

Each of these considerations are discussed in detail below.

DEGRADATION

Understanding eDNA degradation rates in aquatic habitat types is informative when determining presence of target species. The published rates of eDNA degradation will vary depending on local conditions, with observed persistence half-lives ranging from less than 1 hour in a lotic system (i.e., flowing water) up to approximately 58 days under controlled laboratory conditions, with 7 to 25 days being the accepted mid-range. As eDNA tends to degrade in oxygenated and sunlit surface waters, results that confirm eDNA of a target species imply a recent presence, as well as proximity within the aquatic environment.

An emerging area of interest is eDNA preservation in different layers of aquatic ecosystems. Various studies suggest eDNA is better preserved in anoxic environments, such as organic-rich sediment deposits. In theory, this suggests an ability to assess recent presence (including seasonal habitat use) of a target species, based on water samples, and longer-term past presence, based on sediment samples.

WATER FLOWS AND MIXING RATES

Understanding transport of eDNA in both lentic (still water) and lotic systems (flowing water) is also a key component of survey design. System hydrodynamics are considered: large, open, aquatic systems with short water residence-time and rapid mixing (i.e., lotic systems) require different interpretative approaches relative to small, semi-enclosed water bodies with limited exchange (i.e., lentic systems).

6. eDNA filtration equipment

STANDARDIZED FIELD SURVEY PROTOCOLS

Hemmera scientists have learned a number of lessons, since 2014, through implementation of eDNA methods to achieve greater potential gains. Hemmera has been working closely with our university research partners and regulatory agencies to:

- advance field and laboratory standards and procedures critical to data accuracy
- develop provincial standards for field survey methods for British Columbia
- develop laboratory quality assurance methods and practices to improve accuracy of primer development and validation.

DETECTION RELIABILITY

The utility of eDNA survey methods now and in the future requires adequate confidence in the reliability of study results and interpretation by agencies, stakeholders, and the public. Hemmera, with our research collaborators at the University of Victoria and Bureau Veritas (a global commercial analytical laboratory), set out in 2016 to make appropriately validated and certified targeted species eDNA laboratory analytical services widely available to all interested parties, and to introduce greater rigour into the field and laboratory aspects of eDNA studies. With a research grant from Innovate BC, we successfully introduced a commercially available set of eDNA tests, through Bureau Veritas, in 2018. Through collaboration with the Canadian Standards Association and others, we are working to establish a common set of standards for eDNA based ecological surveys, to ensure consistency in transparency, reproducibility, and reliability of eDNA studies to supplement or replace traditional ecological surveys.

ATTITUDINAL CHANGE

Just as in courtroom dramas, skepticism about the reliability of eDNA results within the professional and regulatory community is widespread. Surprisingly, resistance to the use of eDNA has persisted despite repeated demonstration of poor efficacy (i.e., low detection probabilities) of traditional survey techniques. Hemmera has been instrumental in developing and implementing improved field and laboratory practices and procedures to limit analytical errors (i.e., false positives and false negatives) and thereby, directly addressing previous limitations of eDNA use. Communicating these successes and the reliability, efficiency, and cost benefits of eDNA has been helping to changing skeptics into supporters.

7. eDNA filtration in process

SUMMARY

Overall, the use of eDNA methods has achieved significant gains, and offers great promise, in pragmatic and cost-effective management of aquatic and semi-aquatic species and improved accuracy of information that supports environmental management and mitigation. Hemmera believes that improved information and understanding of species' occurrence and distribution through the use of eDNA will better inform decisions for planning and management of transportation and other major infrastructure projects. Along with our growing expertise with eDNA survey methods, so has our interest in extending the use of and rigour in application of eDNA methods. For transportation projects across Canada, we believe this can be best achieved by increasing awareness of the benefits of using eDNA and understanding the constraints to its use.

References:

Willerslev, E., A.J. Hansen, J. Binladen, et al, 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science 300* (5620): 791-795

Ficetola G.F., C. Miaud, F. Pompanon and P. Taberlet, 2008. Species detection using environmental DNA from water samples. Biology Letters 4: 423-425.