Environmental DNA (eDNA)

A Revolutionary Sampling Technique for Aquatic Ecological Studies

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RemTech

October 14, 2016

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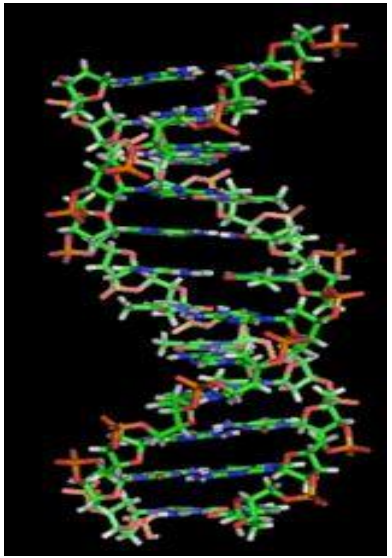
Outline

- What is eDNA
- Methodology / Study Design Considerations
- Efficiencies and Limitations
- Current Applications
- Potential Applications

The Basics

- **Deoxyribonucleic acid** (DNA) molecules carry an organism's genetic information.
- Base pair sequences are unique between organisms: these differences provide a unique way to identify species, populations and individuals.
- The use of mitochondrial DNA is preferred as it’s more abundant than nuclear DNA



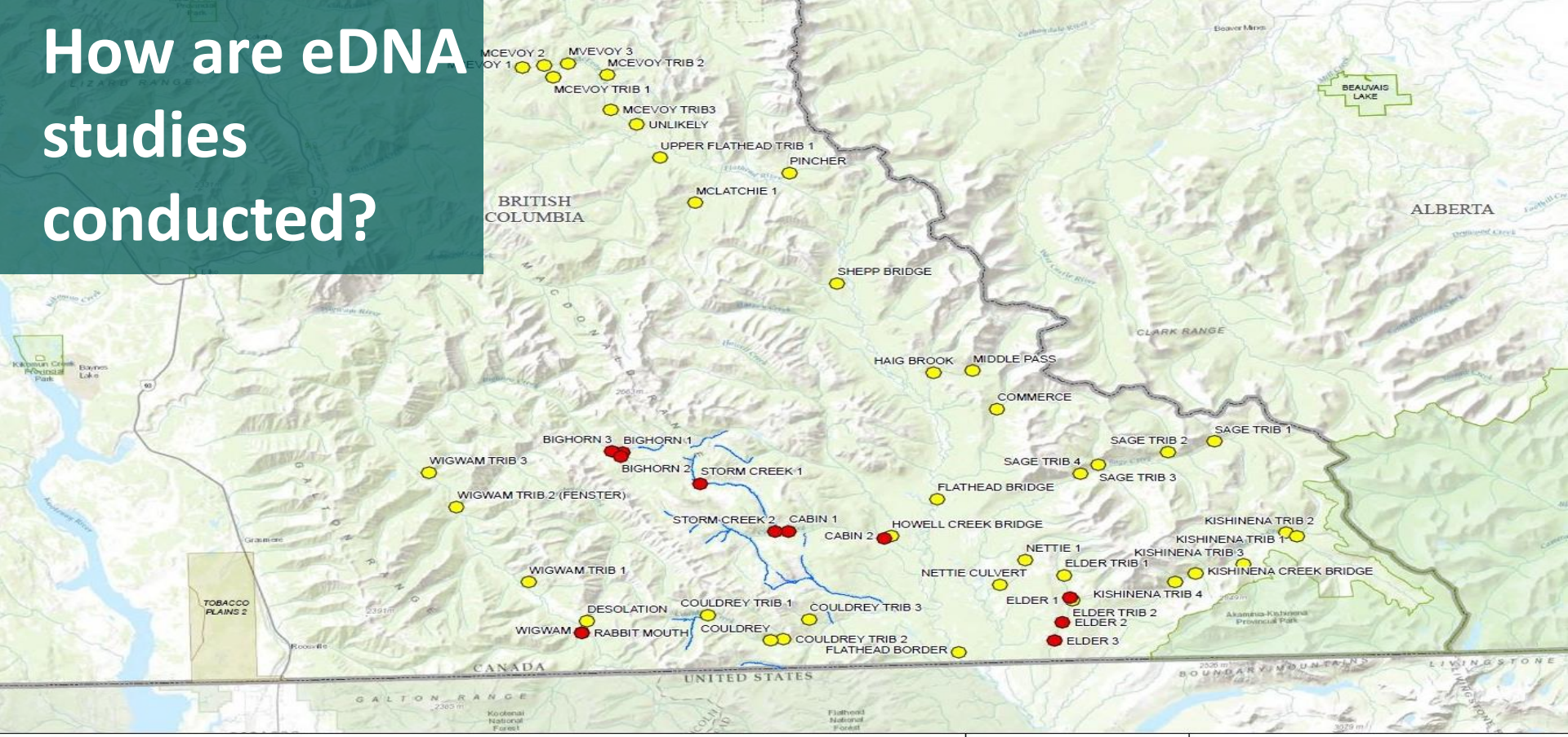
What is eDNA

- **Environmental DNA (eDNA)**

  Relies on the detection of naturally occurring genetic materials that can be collected from the environment, including:

  - gametes
  - dead skin cells
  - feces, urine, saliva
  - egg plasma

How are eDNA studies conducted?



Legend

Rocky Mountain Tailed Frog eDNA Results
- Species Detected
- Species not Detected
- Approved WHA for Rocky Mountain Tailed Frog

- Park or Protected Area
- First Nations Reserve

N

1:275,000

0 5 10

Kilometres

Background Image: ESRI World Topo Map

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CLIENT:
Ministry of Forest, Lands and Natural Resource Operations

ROCKY MOUNTAIN TAILED FROG eDN
Kootenay, BC

ROCKY MOUNTAIN TAILED FROG SAMPLE

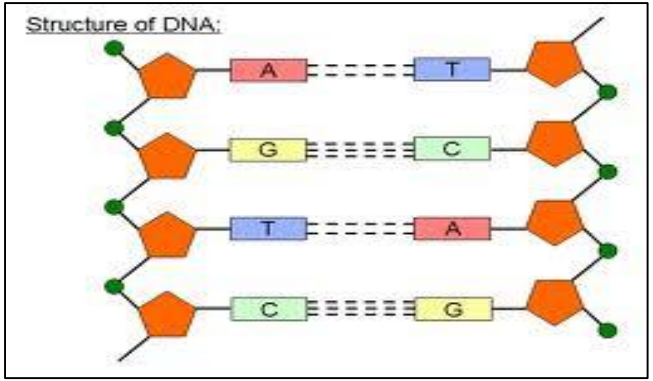
PROJECT No.
1290-022.02

March 2015

Step 1 – Primer Design

A good primer will contain an inclusive consensus sequence that incorporates all within-species variability for a species in a well-known sequence of DNA.

Primers can be reviewed against sequences published in GenBank or against sequences obtained from tissue samples of target and co-occurring closely related species.



- Primers need to incorporate the full range of genetic variation for the target species to avoid false negatives.

- Primers need to incorporate the full range of genetic variation for **closely related, co-occurring species** to avoid false positives.

Step 1 – Primer Design

Primer development requires a comprehensive screening process to exclude sympatric species and prevent false positive detections.

Target Taxa: Red-legged frog

Sympatric Taxa: LICL, LICA, RAPR, PSRE

Step 2 – Sample Collection

Collect water samples from your target system.

Depending on your target species and the habitat types, you need to consider the following:

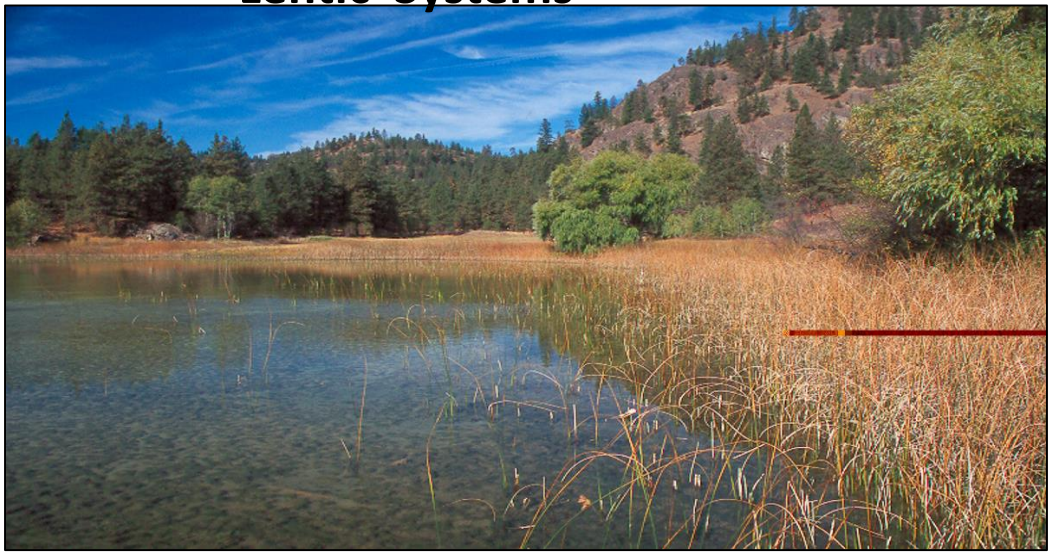
1. Volume of water
2. Number of replicates
3. Collection timing

Survey Design Considerations

Consider sampling requirements for the study area.

Lotic Systems

Lentic Systems



Study Design Considerations

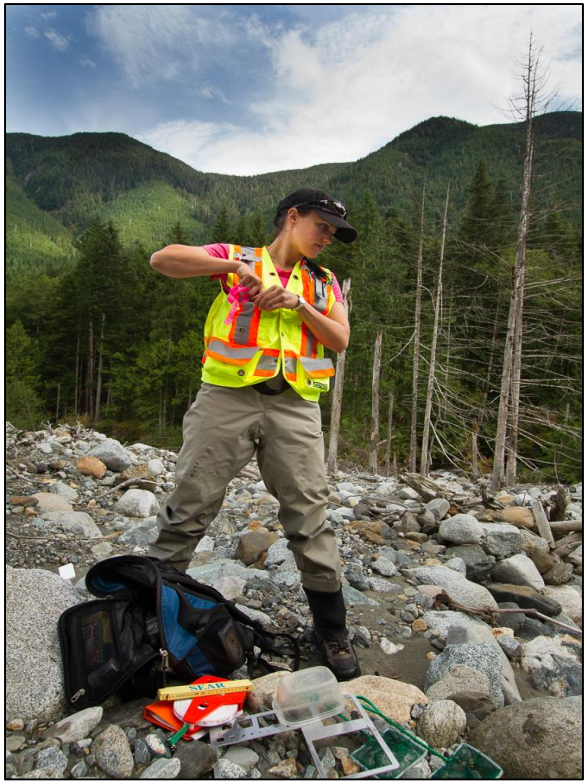
Know the species' life history

- Is there a permanently aquatic life history phase…

- …or does your target taxa tadpole mature in three days, or 6 years?

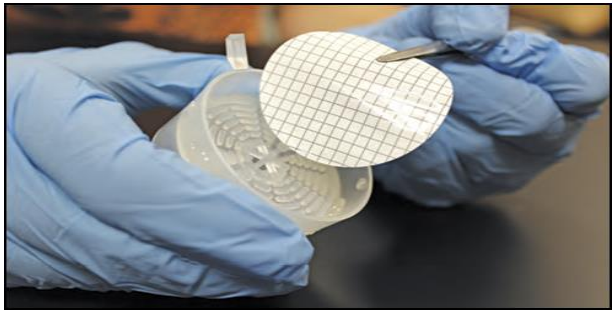
Step 2 – Sample Collection

Collect water samples from the study area.



Step 3 - Filtering

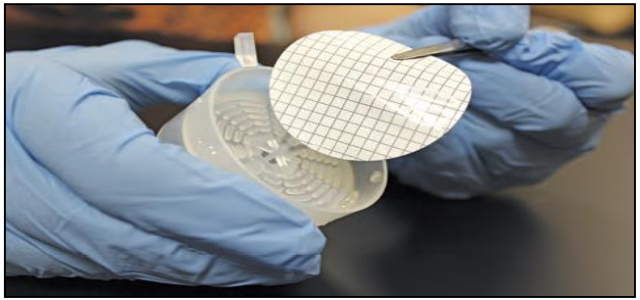
- Unless filtering in the field samples must be stored in a refrigerator prior to filtration.
- Samples are filtered through a membrane using a peristaltic pump.
- Once filtration is complete, the membrane can be frozen or dehydrated in vials with molecular-grade ethanol*.



Step 4 – Genetic Testing

Hemmera works collaboratively with **Dr. Caren Goldberg** – WSU and with **Dr. Caren Helbing** – UVic.

- eDNA extractions and qPCR setups should be conducted in a PCR-free laboratory space where concentrated (such as from tissue) DNA samples have not been handled.

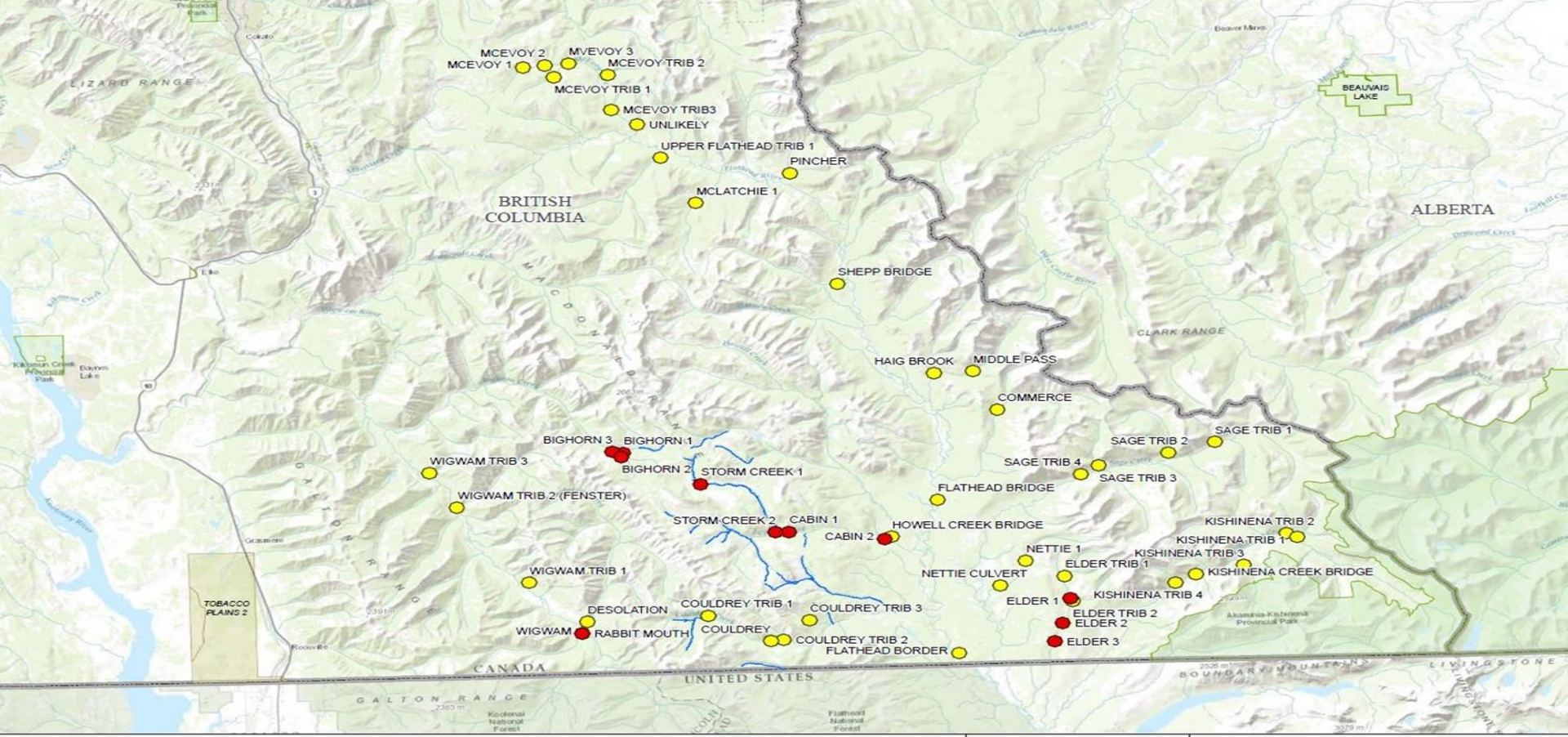


- Thermocyclers and real-time PCR machines should be located outside of this space.

Type 1 & Type 2 Errors

- Type 1 error: false positive detection
- Type 2 error: false negative detection

- **Replicate samples** are required to estimate occupancy while accounting for uncertainty
- Include **known presence sites** in study design to measure efficacy.
- Distilled water can be used (**lab blind-test**) to control for contamination during both the filtering process and during lab-testing.
- Clean field procedures are required to decrease risk of **sample contamination**



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What are the advantages of eDNA?

- More cost effective*
- More accurate
- Less invasive

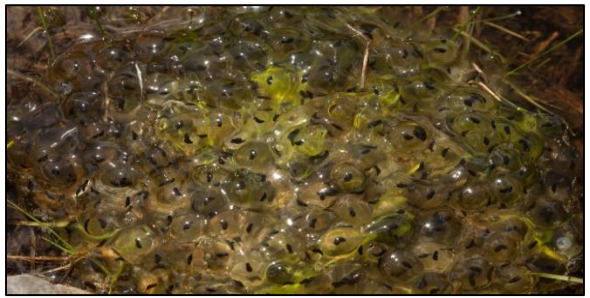
Limitations of eDNA

What eDNA can tell us today (binary answer):

- If the target taxa was present at the site during, or immediately prior to, the time of sampling

What eDNA won't tell us (yet?); Abundance:

- Target taxa abundance and density
- Duration and frequency of habitat use
- Precise physical proximity of target taxa (hard to define transport potential)



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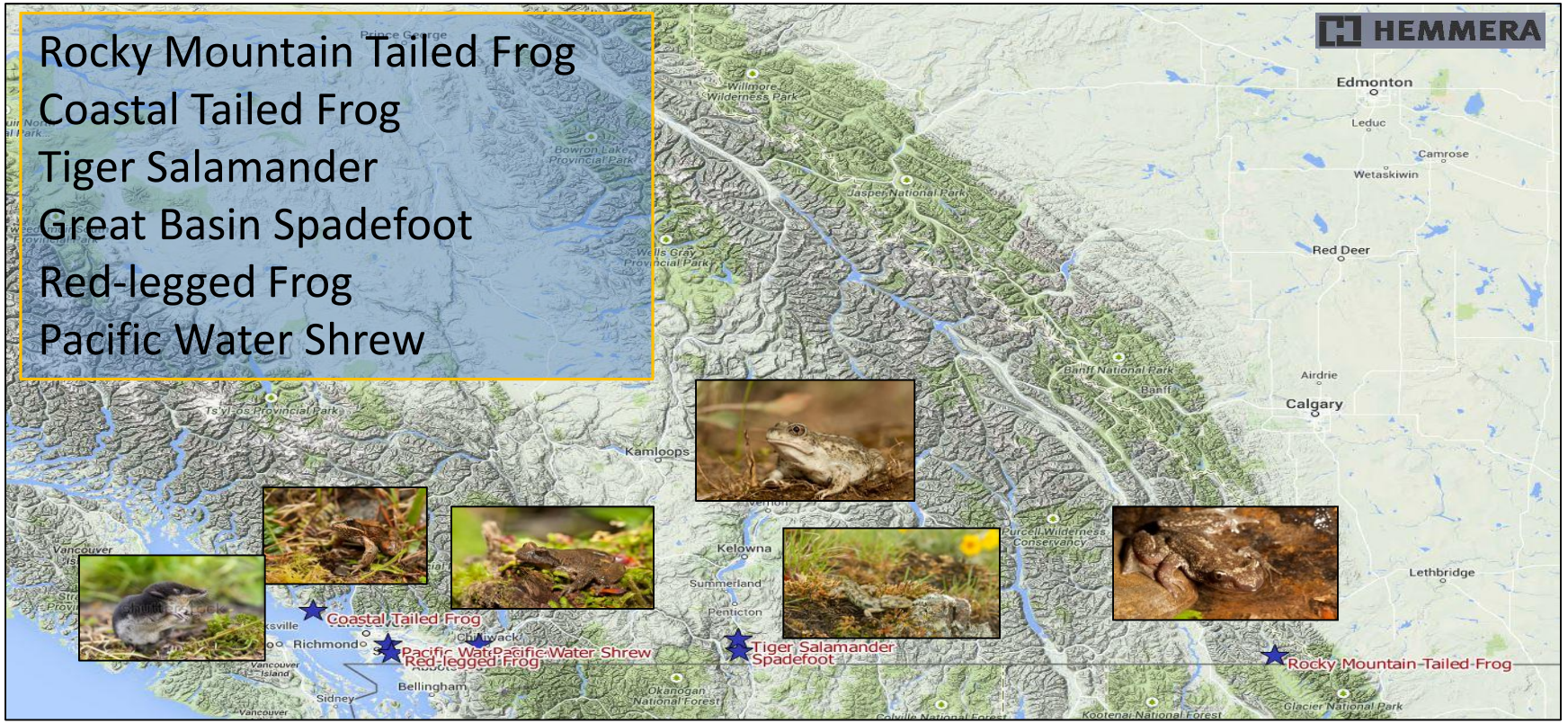
Hemmera and eDNA

Hemmera: Current and Completed BC Projects



Hemmera: Current and Completed Yukon Projects

Yukon researchers unlock way to track salmon by their DNA

Scientists can detect traces of poop, mucus and skin up to 3 weeks after fish swim past

CBC News Posted: Mar 17, 2016 12:28 PM CT | Last Updated: Mar 17, 2016 1:21 PM CT

A Chinook salmon swimming upstream. Yukon researchers have figured out how to track fish up to three weeks after they've passed through an area. (Associated Press)

Bull Trout
Arctic Grayling
Western Toad
Columbia Spotted Frog

Current Alberta eDNA Projects

ALBERTA AMPHIBIAN AND REPTILE SPECIALIST GROUP

11:30 Waterton Lakes National Park Rocky Mountain Tailed Frog eDNA Survey (Kim Pearson, Parks Canada).

11:45 Detection of Three Species of Amphibian in NW Alberta Using eDNA (Kris Kendell, ACA)

12:00 Laboratory Protocols for Detecting Northern Leopard Frog eDNA (Brian Eaton, AI-TF)

Standard Operating Procedures

In 2015 we developed a provincial standard protocol document for the BC Ministry of Environment for application by other eDNA practitioners in BC and beyond.

Current Applications

- eDNA facilitates early detection and monitoring for management of:
  - Species of regulatory concern
  - Pathogens
  - Invasive species

- Useful for early detection of invasive species.

  "Some intensive eradication programs for invasive species fail when a few surviving individuals recolonize the ecosystem. eDNA methods may provide a means of confirmina eradication of all invaders" *(USGS 2012*

Future Expanded Application

Hemmera was the first to apply eDNA in a commercial (non-academic) setting in western Canada, and we're excited by its potential to:

- Inventory for an increasing number of **Species at Risk**
- Assess effectiveness of **restoration** programs
- Support **environmental assessment** processes
- Assess effectiveness of control programs for **invasive species**

Thank you

Contact Us

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Existing Primers

Common name	Scientific name
Amphibians	
Rocky mountain tailed frog	*Ascaphus montanus*
Northern red-legged frog*	*Rana aurora*
Great Basin Spadefoot*	*Spea intermontana*
Tiger salamander*	*Ambystoma mavortium*
Columbia spotted frog	*Rana luteiventris*
Northern leopard frog	*Lithobates pipiens*
Western toad	*Anaxyrus boreas*
Coastal giant salamander	*Dicamptodon tenebrous*
Oregon spotted frog	*Rana pretiosa*
Cascades frog	*Rana cascadia*
Long-toed salamander	*Ambystoma macrodactylum*
Fishes	
Chinook salmon	*Oncorhynchus tshawytscha*
Lake trout	*Salvelinus namaycush*
Bull trout	*Salvelinus confluentus*
Brook trout	*Salvelinus fontinalis*
Mammals	
Pacific Water Shrew*	*Sorex benderii*

** Hemmera developed and maintains IP.*

Requested Primers

Common name	Scientific name
Fishes	
Dolly Varden	*Salvelinus malma*
Least Cisco	*Coregonus sardinella*
Pygmy whitefish	*Prosopium coulterii*
Arctic grayling	*Thymallus arcticus*
Aquatic Invasives of Concern	
Didymo	*Didymosphenia geminata*
Zebra (dreissenid) mussels	*Dreissenidae*
New Zealand Mud Snail	*Potamopyrgus antipodarum*
Water Weeds	*Elodea spp.*
Eurasian Milfoil	*Myriophyllum spicatum*
VHSV	*Viral hemorrhagic septicemia virus*
Myxosporean parasite	*Myxobolus cerebralis*
Fanwort	*Cabomba sp*
Spiny Water Flea	*Bythotrephes longimanus*
Goldfish	*Carassius auratus auratus*
Rainbow Trout	*Oncorhynchus mykiss*
Arctic Char	*Salvelinus alpinus*
Threespine Stickleback	*Gasterosteus aculeatus*
Silver Carp	*Hypophthalmichthys molitrix*
Northern Snakehead	*Channa argus*
Rusty Crayfish	*Orconectes rusticus*
Invertebrates	
Rocky Mountain Ridged Mussel	*Gonidea angulata*

When to use eDNA: CBA

CBA that neglects full consideration of each of these methodological attributes may be misleading.

Attribute	Conventional Methods	eDNA
Efficacy	Low-High	High
Multi-species	No	Yes
Retro-active addition of taxa	No	Yes
Adaptive design/testing	No	Yes
Observer and detectability bias	High	Low
Permitting required	Yes	No
Invasiveness	High	Low
Pathogen transfer risk	High	Low
Timing	Restrictive	Less Restrictive
Special equipment/training	Medium-high	Low
Safety considerations	Medium-high	Low
Abundance and proximity	Yes (with appropriate design)	No