Commercial Environmental DNA (eDNA) Assays: Sampling, Analysis and Data Interpretation

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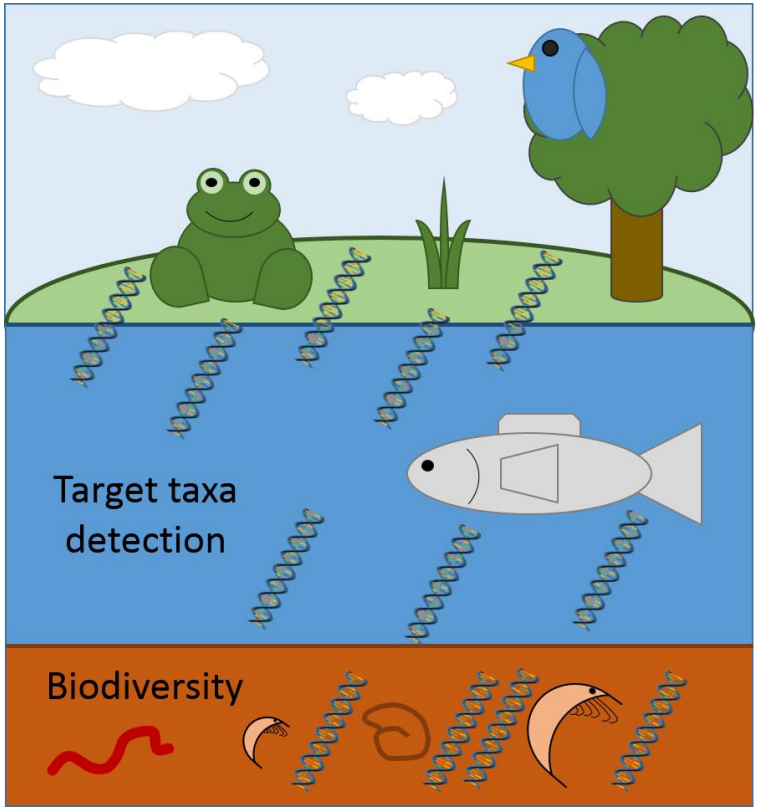
Outline

- eDNA Definition & Current Status
- Advantages & Limitations
- Considerations for Sampling & Testing
- Results Interpretation
- Commercial eDNA assays
- Case Study – Rocky Mountain Tailed Frog

Environmental DNA (eDNA)

- DNA released to the environment
- Collected in water/sediment/soil
- Isolate DNA and analyze for presence of target species
- Environmental assessments:
  - Is the target species present?



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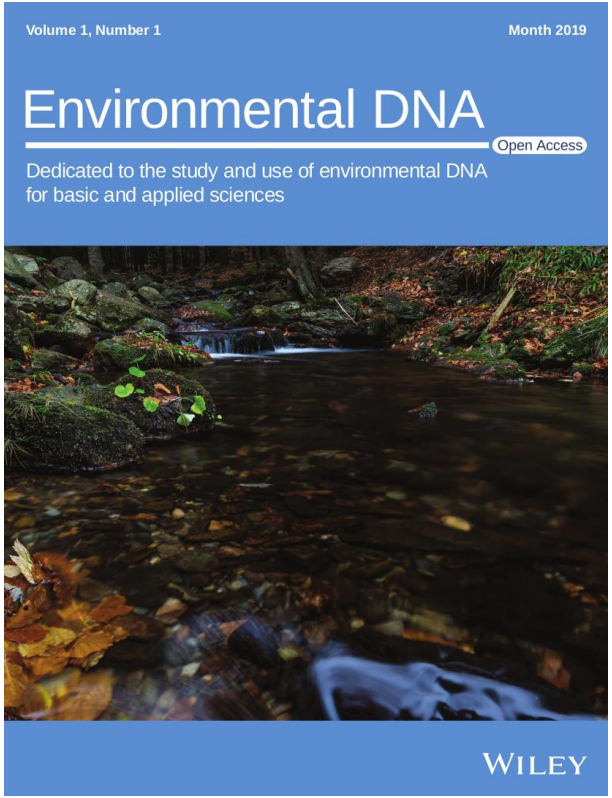
Does eDNA testing really work?

- It works! – like CSI for wildlife
- First eDNA report 2008 (Ficetola *et al*.)
- Today:
  - > 2,500 peer reviewed eDNA articles, 50% within last 4 years
  - Dedicated eDNA conferences:

PISCeS

Pathway to **I**ncrease **S**tandards and **C**ompetency of **e**DNA **S**urveys (University of Guelph, 2018)



New (2019) *Environmental DNA* journal

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eDNA advantages

- **Less invasive** – to habitat and study organisms
- **More sensitive** – can detect low amounts of eDNA
- **Time savings** – quick sample collection
- **Cost effective** – multiple species detection
- **Accurate** – no need for qualified expert in the field
- **Reduced observer bias** – simple sampling
- **Retroactive testing** – archive eDNA samples
- **Ease of access** – no permit required
- **Expanded survey window** – e.g. frog calling

Traditional methods

Vs.

eDNA sampling

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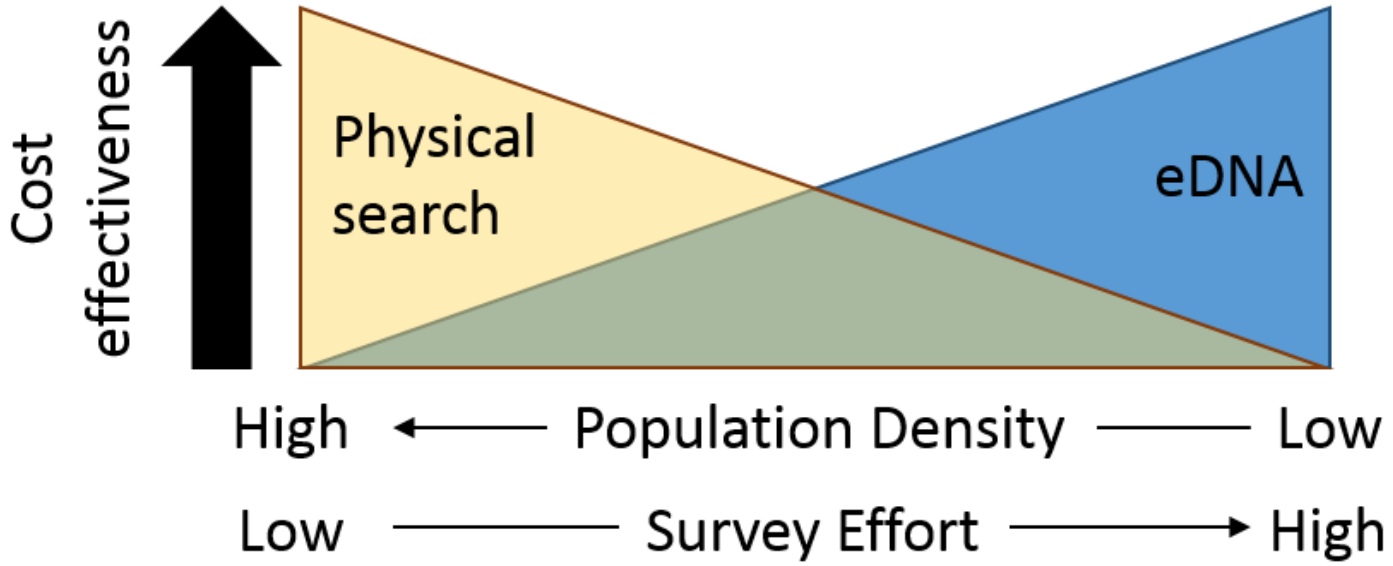
When to use eDNA over traditional methods



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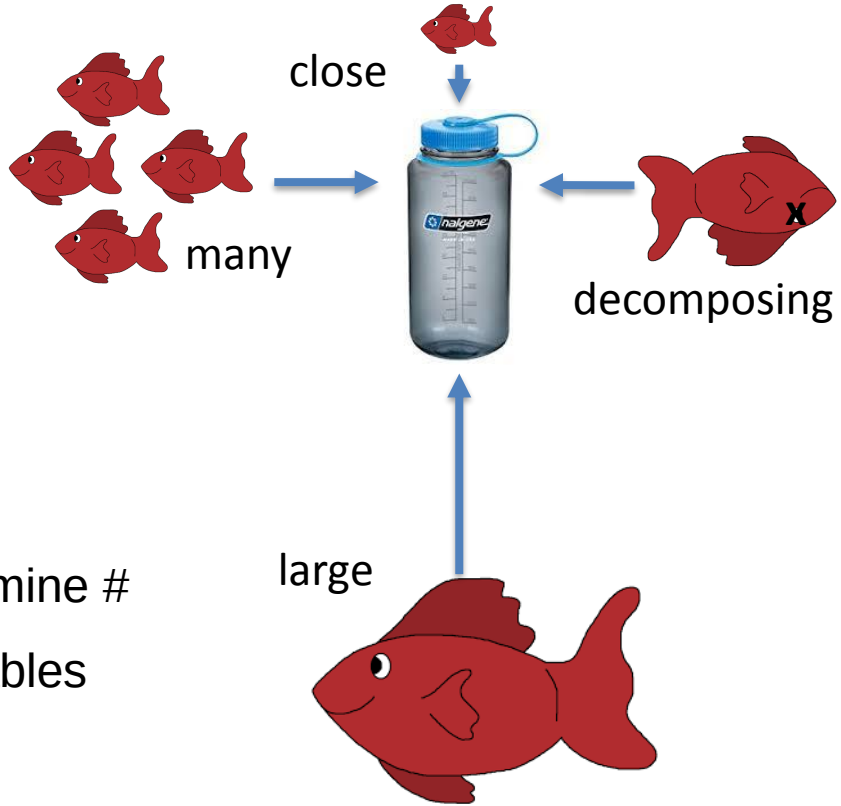
Limitations of eDNA testing

- **Only detects presence of target DNA**
  - X alive or dead?
  - X age?
  - X size?
  - X gender?
  - X breeding status?
  - X exact geographical location?
- **Detect/Non-detect result** – cannot determine #
- **eDNA degradation** – environmental variables

Can eDNA determine # of individuals?



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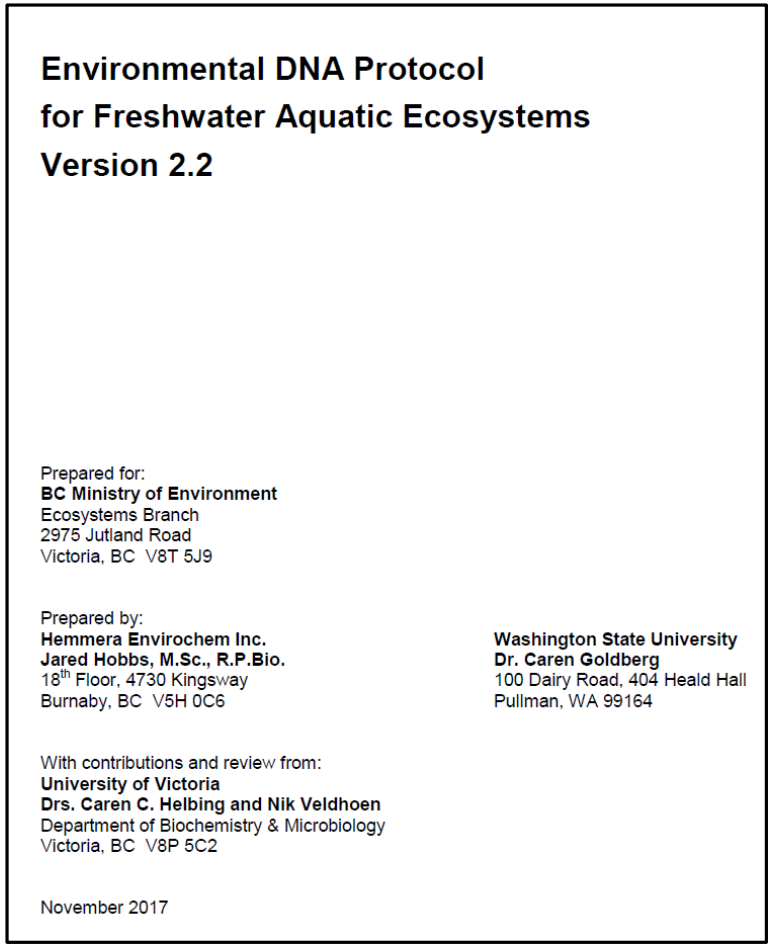
Sampling considerations

- ✓ Time of year species will be present
- ✓ When the species is most active
- ✓ Lifecycle stage that introduces most eDNA
- ✓ Habitat – sample preferred micro-habitats
- ✓ Sample replicates – improves detection



Environmental DNA Protocol
for Freshwater Aquatic Ecosystems
Version 2.2

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November 2017

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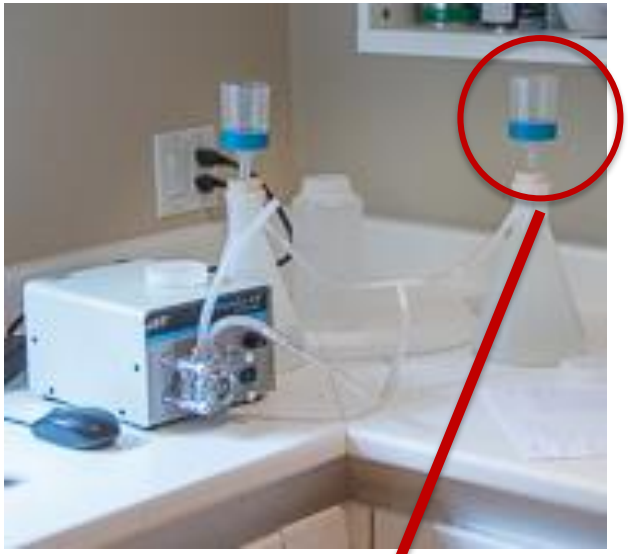
Filtration & preservation

- **Water sample filtration:**
  - ✓ Avoid collecting debris that will clog the filter
  - ✓ Keep the sample on ice
  - ✓ Filter & preserve within 24-hours of collection
  - ✓ Include negative control (distilled water)
- **Sample preservation:**
  - ✓ Silica desiccant preferred over ethanol
- **Submit samples to lab:**
  - ✓ Test as soon as possible for best results



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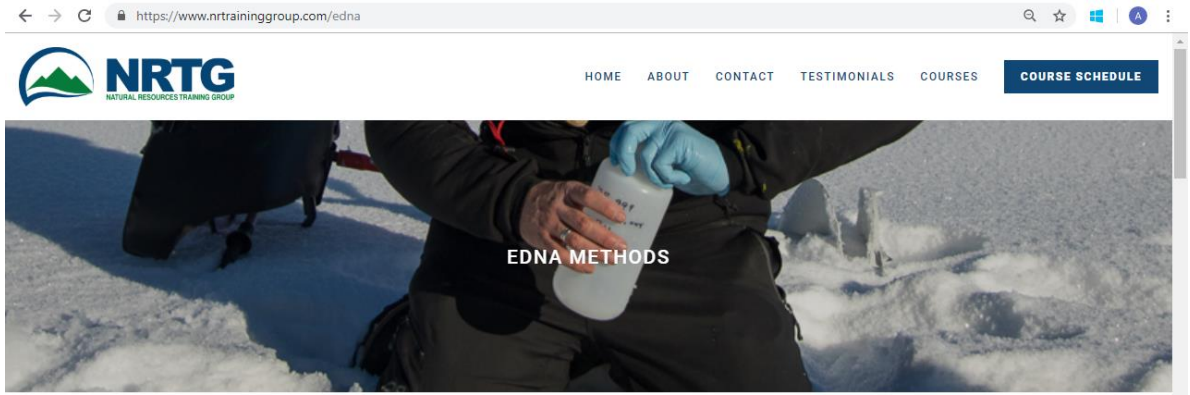
Sampling instruction

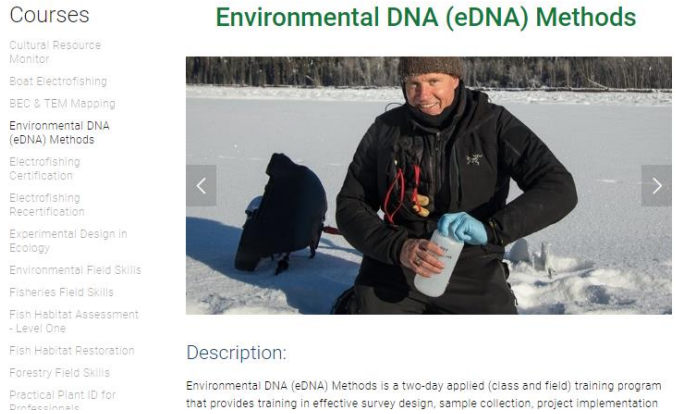
eDNA Methods course -
Natural Resources Training Group

www.nrtraininggroup.com



Jared Hobbs;

Experience with > 50 eDNA projects



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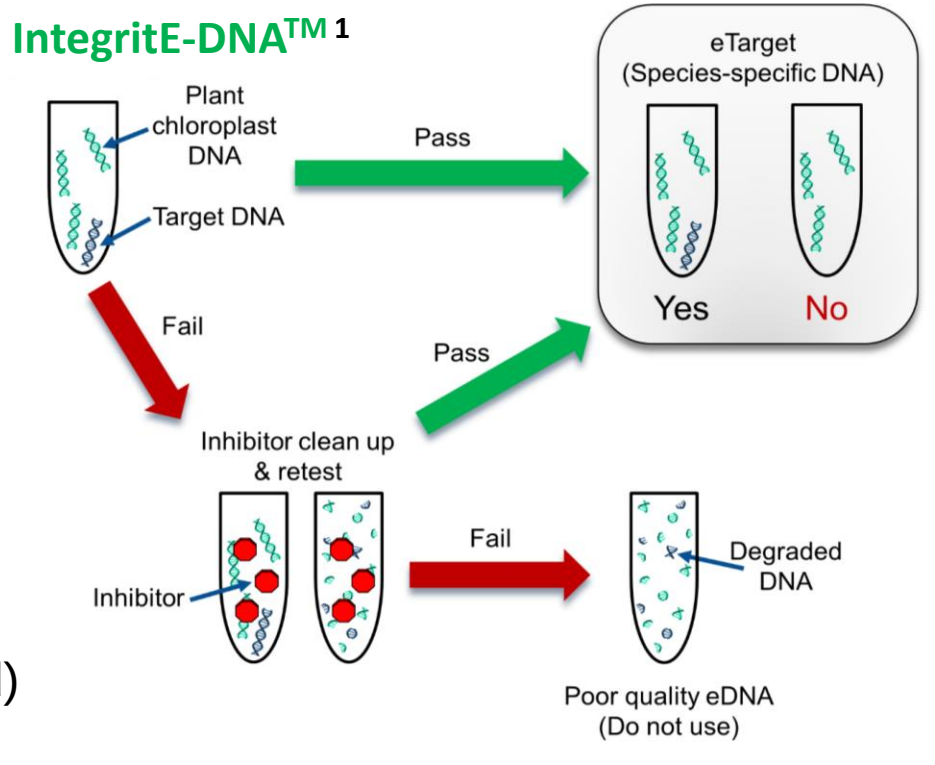
eDNA assay considerations

- **Assay design and validation**
  - ✓ Specificity check -
    - ✓ sympatric species & human DNA
  - ✓ Sensitivity check (LOD)
  - ✓ Validate with field samples
  - ✓ Interlaboratory verification
- **Check for false negatives**
  - eDNA degrades upon release
  - Inhibitors (e.g. humic & tannic acid)



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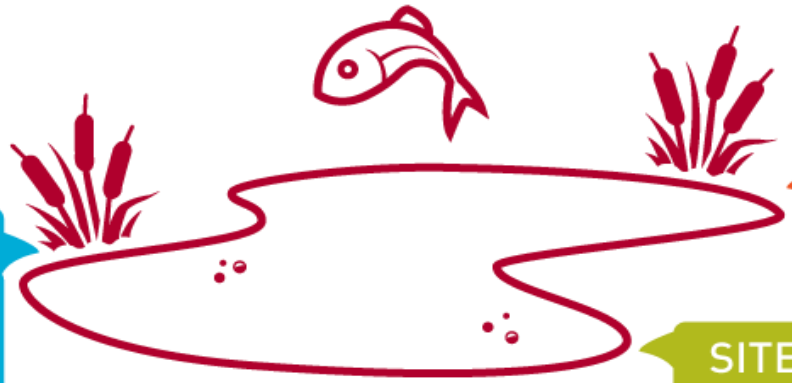
[1] IntegritE-DNA™ – University of Victoria

eDNA results interpretation



SITE A

Sample	Technical Replicate Results	Result
1	+ + + + + + + +	Detect
2	+ + + + + - - -	Detect
3	+ + + + + + + -	Detect
Interpretation	Species Detected	

SITE B

Sample	Technical Replicate Results	Result
1	- - - - - - - -	Non-detect
2	- - - - - - - -	Non-detect
3	+ + - - - - - -	Non-detect
Interpretation	Species Not Detected	

SITE C

Sample	Technical Replicate Results	Result
1	+ + - - - - - -	Non-detect
2	+ + - - - - - -	Non-detect
3	+ - - - - - - -	Non-detect
Interpretation	Species Presence Possible*	

*Additional sampling/testing recommended

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Need for commercial eDNA testing

- R&D focus at university and government labs
- Commercial eDNA service:
  - ✓ Robust
  - ✓ Reproducible
    - ✓ Inter-laboratory verification
  - ✓ Turnaround time to meet industry needs
  - ✓ Quality program that meet ISO 17025
- 2017 BC Innovation Council – Ignite grant
  - University of Victoria, Hemmera Envirochem, Maxxam Analytics

BC INNOVATION COUNCIL

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eDNA Case Study:

Rocky Mountain Tailed Frog

Rocky Mountain Tailed Frog

- Listed Species at Risk in British Columbia
- “Tail" is an extension of the cloaca in males
- Tailed frogs = only frog species that use internal fertilization in North America
- Range is limited to Montana, Idaho, Washington, Oregon, northern California and southeastern British Columbia.

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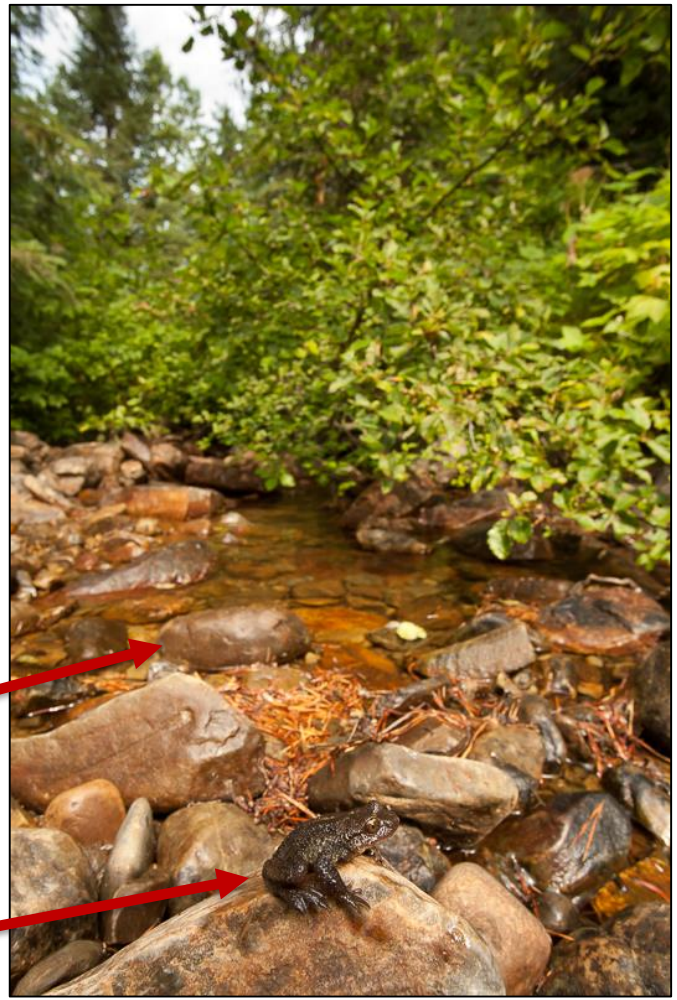
Ideal species for eDNA

- **Habitat** – steep, fast flowing mountain streams difficult to survey by traditional methods
- **Doesn't vocalize** – cannot use audio surveys
- **Eggs hidden** – laid under large rocks
- **Low population density** – difficult to observe

Egg mass

Frog



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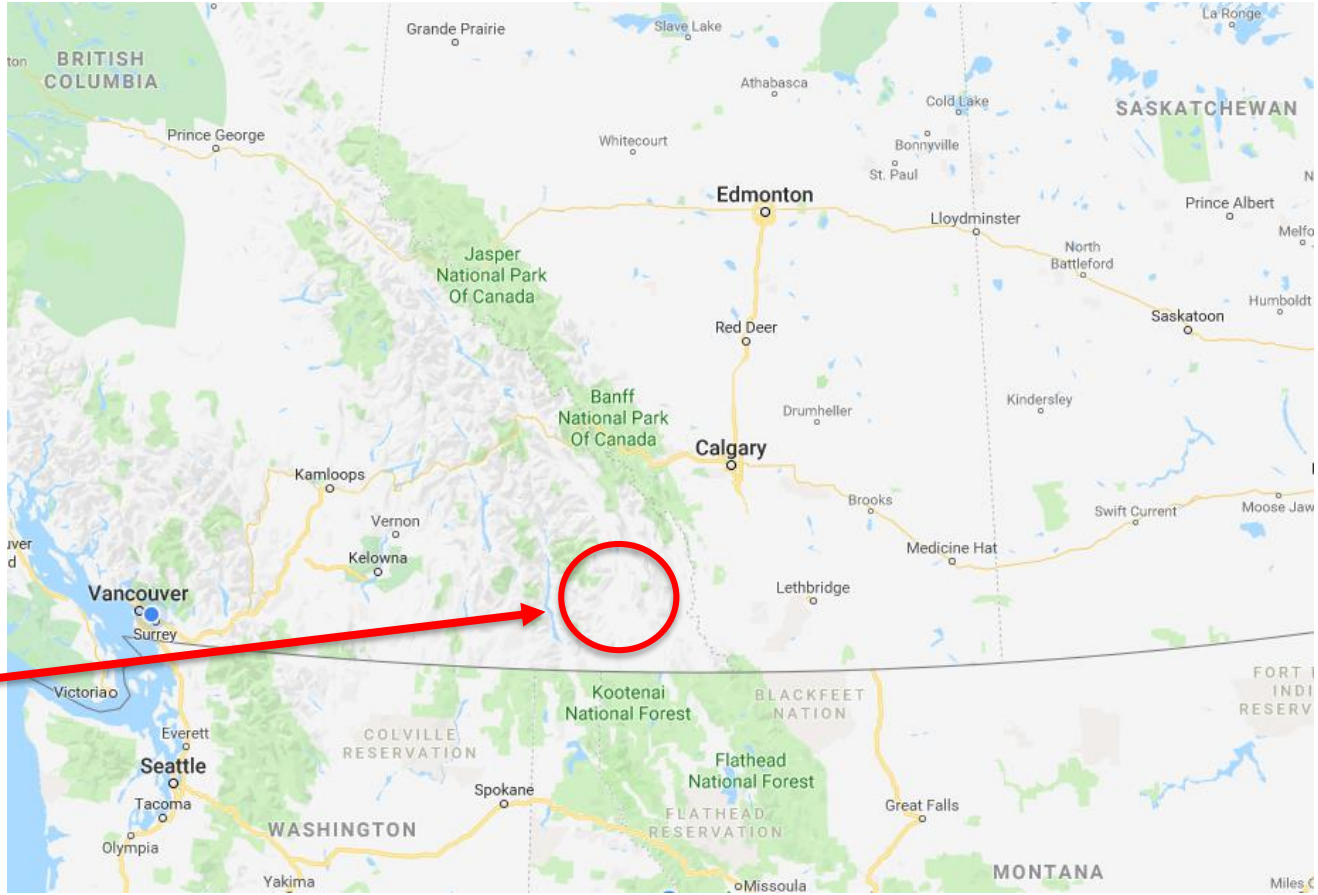
eDNA survey

- **10 sites**
- **2 sample replicates/site**

Survey area:

- **Yahk & Flathead drainage basins**



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Interlaboratory comparison

- 10 sites: A - J
- 2 sample replicates/site
- 20 samples total

Site	Sample #	eDNA Clean Up Required?		IntegritE-DNA Pass?		Rocky Mtn Tailed Frog eDNA Assay	
		Maxxam	UVic	Maxxam	UVic	Maxxam	UVic
A	1	Y	Y	Y	N	1/8	0/8
	2	Y	Y	Y	Y	0/8	0/8
B	3	N	Y	Y	Y	0/8	0/8
	4	Y	Y	Y	Y	1/8	1/8
C	5	N	N	Y	Y	0/8	0/8
	6	N	N	Y	Y	0/8	0/8
D	7	N	Y	N	N	0/8	0/8
	8	N	Y	N	N	0/8	0/8
E	9	N	N	Y	Y	0/8	0/8
	10	N	N	Y	Y	0/8	0/8
F	11	N	N	Y	Y	8/8	8/8
	12	N	N	Y	Y	5/8	5/8
G	13	N	N	Y	Y	7/8	6/8
	14	N	N	Y	Y	3/8	5/8
H	15	N	Y	Y	Y	0/8	0/8
	16	N	N	Y	Y	0/8	0/8
I	17	N	N	Y	Y	0/8	0/8
	18	N	N	Y	Y	0/8	0/8
J	19	N	N	Y	Y	0/8	0/8
	20	N	N	Y	Y	0/8	0/8

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Inter-laboratory comparison

- DNA clean-up required:
- Maxxam: 25% samples
- UVic: 35% samples

Site	Sample #	eDNA Clean Up Required?		IntegritE-DNA Pass?		Rocky Mtn Tailed Frog eDNA Assay	
		Maxxam	UVic	Maxxam	UVic	Maxxam	UVic
A	1	Y	Y	Y	N	1/8	0/8
	2	Y	Y	Y	Y	0/8	0/8
B	3	N	Y	Y	Y	0/8	0/8
	4	Y	Y	Y	Y	1/8	1/8
C	5	N	N	Y	Y	0/8	0/8
	6	N	N	Y	Y	0/8	0/8
D	7	Y	Y	N	N	0/8	0/8
	8	Y	Y	N	N	0/8	0/8
E	9	N	N	Y	Y	0/8	0/8
	10	N	N	Y	Y	0/8	0/8
F	11	N	N	Y	Y	8/8	8/8
	12	N	N	Y	Y	5/8	5/8
G	13	N	N	Y	Y	7/8	6/8
	14	N	N	Y	Y	3/8	5/8
H	15	N	Y	Y	Y	0/8	0/8
	16	N	N	Y	Y	0/8	0/8
I	17	N	N	Y	Y	0/8	0/8
	18	N	N	Y	Y	0/8	0/8
J	19	N	N	Y	Y	0/8	0/8
	20	N	N	Y	Y	0/8	0/8

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Inter-laboratory comparison

- IntegritE-DNA:
- Sample 1 – x UVic
- Site D – x Maxxam & UVic

Site	Sample #	eDNA Clean Up Required?		IntegritE-DNA Pass?		Rocky Mtn Tailed Frog eDNA Assay	
		Maxxam	UVic	Maxxam	UVic	Maxxam	UVic
A	1	Y	Y	Y	N	1/8	0/8
	2	Y	Y	Y	Y	0/8	0/8
B	3	N	Y	Y	Y	0/8	0/8
	4	Y	Y	Y	Y	1/8	1/8
C	5	N	N	Y	Y	0/8	0/8
	6	N	N	Y	Y	0/8	0/8
D	7	Y	Y	N	N	0/8	0/8
	8	Y	Y	N	N	0/8	0/8
E	9	N	N	Y	Y	0/8	0/8
	10	N	N	Y	Y	0/8	0/8
F	11	N	N	Y	Y	8/8	8/8
	12	N	N	Y	Y	5/8	5/8
G	13	N	N	Y	Y	7/8	6/8
	14	N	N	Y	Y	3/8	5/8
H	15	N	Y	Y	Y	0/8	0/8
	16	N	N	Y	Y	0/8	0/8
I	17	N	N	Y	Y	0/8	0/8
	18	N	N	Y	Y	0/8	0/8
J	19	N	N	Y	Y	0/8	0/8
	20	N	N	Y	Y	0/8	0/8

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Inter-laboratory comparison

- Rocky Mountain Tailed Frog eDNA assay

Site	Sample #	eDNA Clean Up Required?		IntegritE-DNA Pass?		Rocky Mtn Tailed Frog eDNA Assay	
		Maxxam	UVic	Maxxam	UVic	Maxxam	UVic
A	1	Y	Y	Y	N	1/8	0/8
	2	Y	Y	Y	Y	0/8	0/8
B	3	N	Y	Y	Y	0/8	0/8
	4	Y	Y	Y	Y	1/8	1/8
C	5	N	N	Y	Y	0/8	0/8
	6	N	N	Y	Y	0/8	0/8
D	7	Y	Y	N	N	0/8	0/8
	8	Y	Y	N	N	0/8	0/8
E	9	N	N	Y	Y	0/8	0/8
	10	N	N	Y	Y	0/8	0/8
F	11	N	N	Y	Y	8/8	8/8
	12	N	N	Y	Y	5/8	5/8
G	13	N	N	Y	Y	7/8	6/8
	14	N	N	Y	Y	3/8	5/8
H	15	N	Y	Y	Y	0/8	0/8
	16	N	N	Y	Y	0/8	0/8
I	17	N	N	Y	Y	0/8	0/8
	18	N	N	Y	Y	0/8	0/8
J	19	N	N	Y	Y	0/8	0/8
	20	N	N	Y	Y	0/8	0/8

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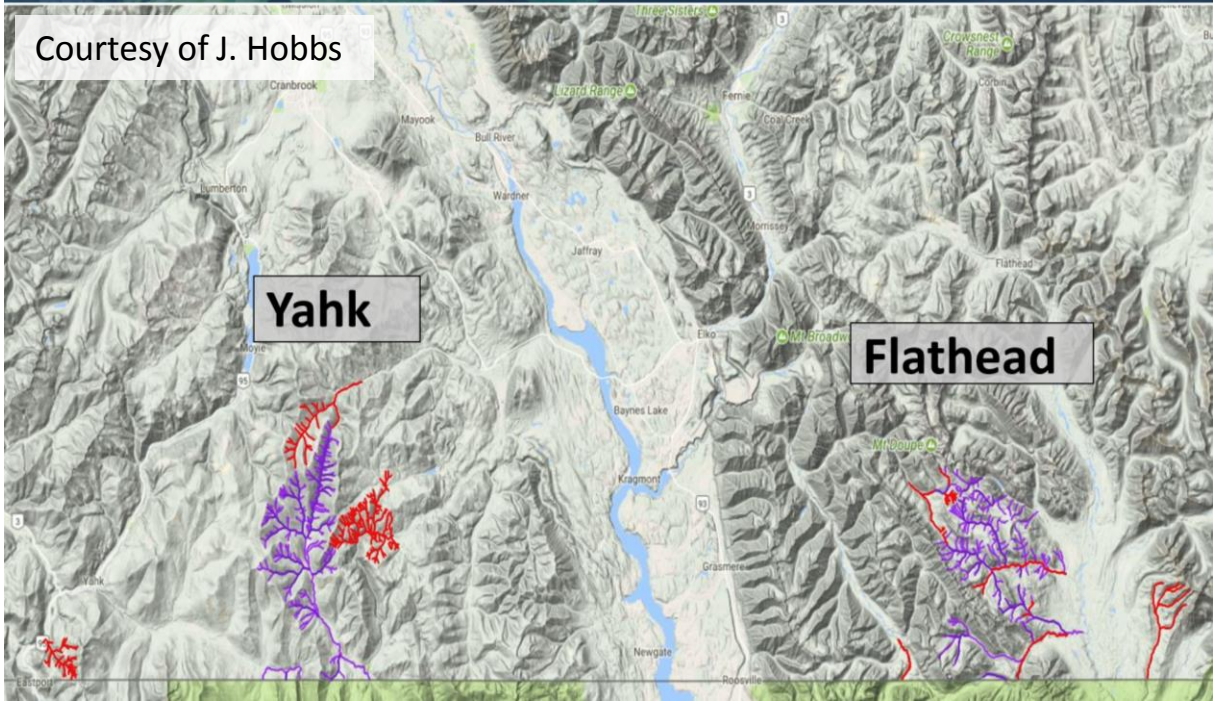
Current Canadian Distribution



eDNA expanded the distribution for Rocky Mountain Tailed Frog

Purple water courses = traditional methods over 8 years: intensive & costly

Red water courses = 4 new streams identified using eDNA at low cost over 2 years

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- Maxxam has 16 eDNA assays available
- Develop eDNA assays for additional species

AVAILABLE eDNA ASSAYS

	eDNA TEST	SPECIES	COMMON NAME
FROG	RAAU	*Rana aurora*	Northern red-legged frog
	ASMO	*Ascaphus montanus*	Rocky mountain tailed frog
	LICA	*Lithobates (Rana) catesbeiana*	North American bullfrog
	ANBO	*Anaxyrus (Bufo) boreas*	Western toad
	RAPR	*Rana pretiosa*	Oregon spotted frog
	ASTR	*Ascaphus truei*	Pacific (Coastal) tailed frog
FISH	ONTS	*Oncorhynchus tshawytscha*	Chinook salmon
	ONKI	*Oncorhynchus kisutch*	Coho salmon
	THAR	*Thymallus arcticus*	Arctic grayling
	ONCL	*Oncorhynchus clarkii*	Cutthroat trout
	ONMY	*Oncorhynchus mykiss*	Rainbow trout (Steelhead trout)
	ONNE	*Oncorhynchus nerka*	Sockeye salmon
	eFish	*Oncorhynchus nerka* (ONNE)	Sockeye salmon
		Oncorhynchus gorbuscha (ONGO)	Pink salmon
		Oncorhynchus keta (ONKE)	Chum salmon
		Thymallus arcticus (THAR)	Arctic grayling
		Oncorhynchus clarkii (ONCL)	Cutthroat trout
		Oncorhynchus mykiss (ONMY)	Rainbow trout
		Oncorhynchus tshawytscha (ONTS)	Chinook salmon
		Oncorhynchus kisutch (ONKI)	Coho salmon
		Salmo salar (SASA)	Atlantic salmon
		Salvelinus malma (SAMA)	Dolly Varden
		Prosopium cylindraceum (PRCY)	Round whitefish
		Cottus cognatus (COCO)	Slimy sculpin
OTHER	AMMV (AMTI)	*Ambystoma mavortium*	Blotched tiger salamander
		Ambystoma tigrinum	Tiger salamander
	SOBE	*Sorex bendirii*	Pacific water shrew

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Acknowledgements

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