Environmental DNA (eDNA)

A Revolutionary Sampling Technique for Aquatic Ecological Studies

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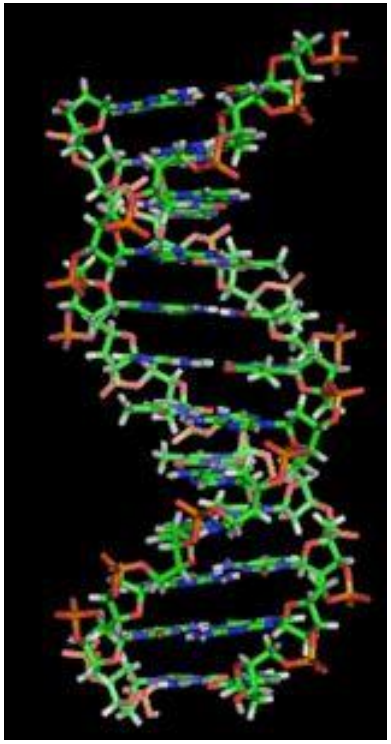
Outline

- What is eDNA
- Methodology
- Considerations (timing, feasibility)
- Efficiencies and limitations
- Current Applications
  - Hemmera – current projects and experience
- 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.
- With eDNA, the use of mitochondrial DNA is preferred –it's more abundant than nuclear DNA and Genbank has more sequence data.



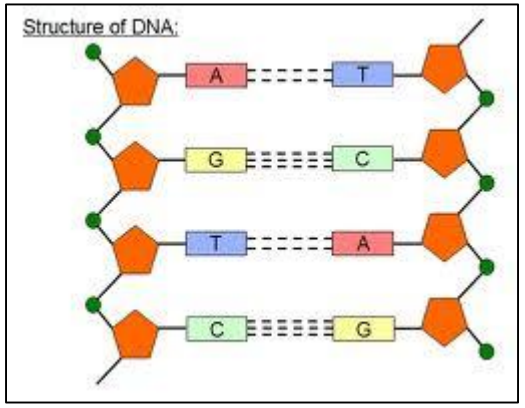
What is eDNA

- **Environmental DNA (eDNA)**
  - Relies on the detection of naturally occurring genetic materials that can be collected from the environment
  - gametes, dead skin cells, feathers, hair, feces, urine, egg plasma, saliva

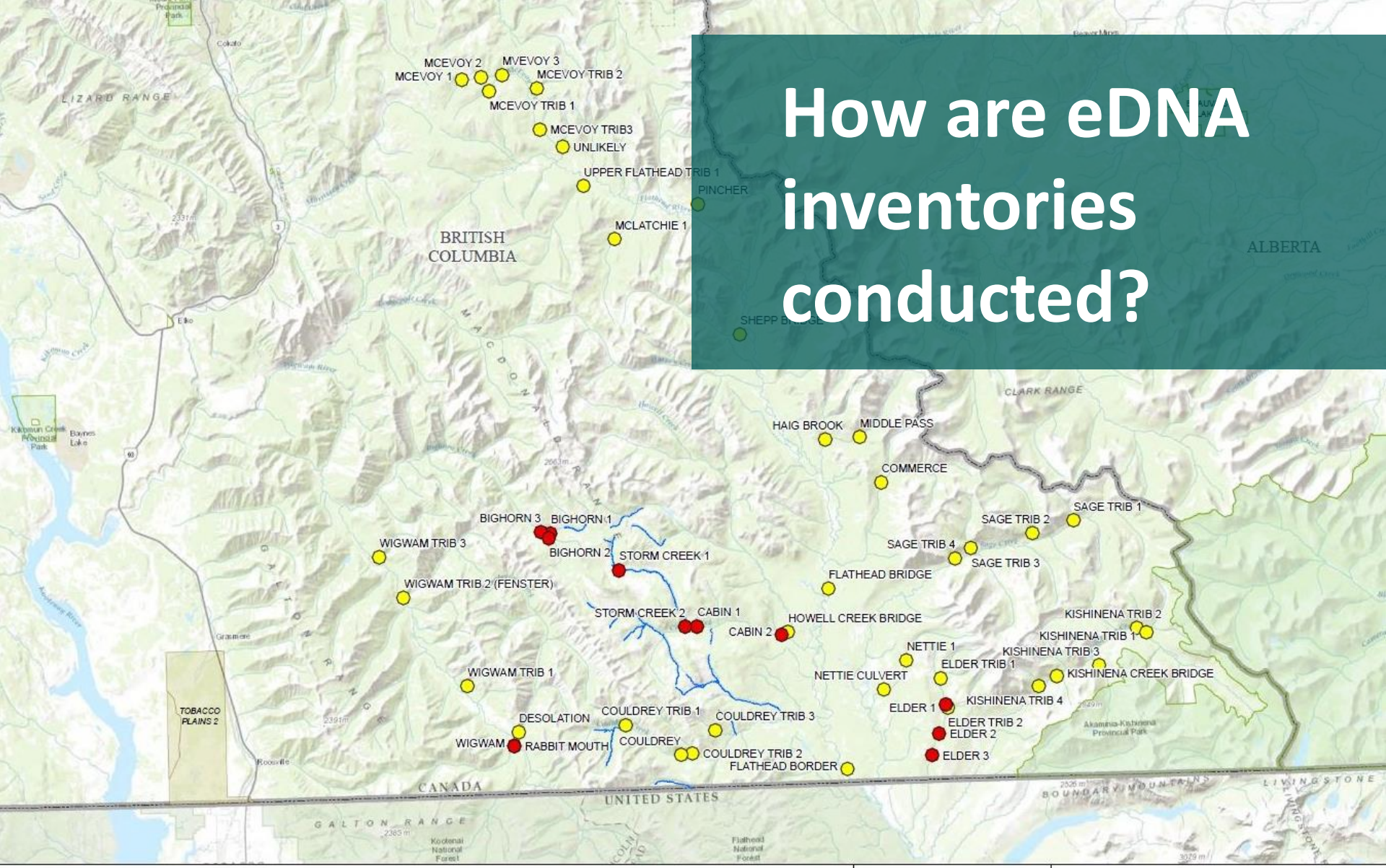
Primer and Probe Design

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.



How are eDNA inventories 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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ROCKY MOUNTAIN TAILED FROG eDN
Kootenay, BC

ROCKY MOUNTAIN TAILED FROG SAMPLE

CLIENT:
Ministry of Forest, Lands and Natural Resource Operations

PROJECT No.
1290-022.02

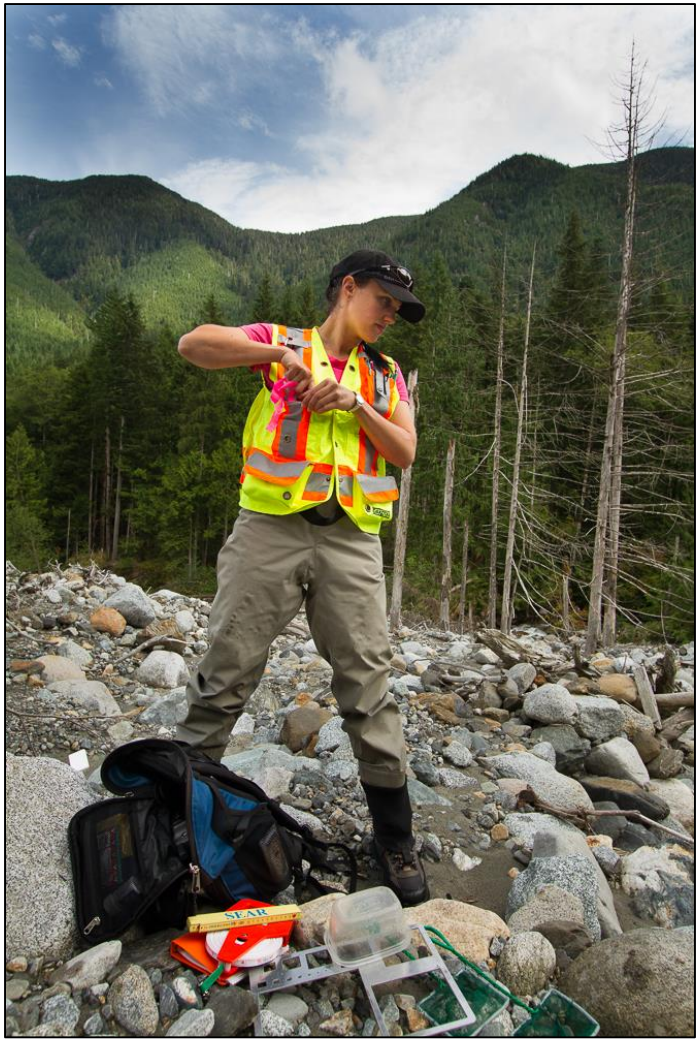
March 2015

Field Sampling Methodology – Step 1

Four methods for field sampling have been developed to date:

1. Collection of 15 mL of water which is then preserved using ethanol and sodium acetate. Then frozen immediately
2. Filter water through a glass fiber filter.
3. Filter water through carbonate filter.
4. **Filter water through a cellulose nitrate filter.**

Option #4 allows off-site filtering and is the most practical method for field use.



Field Filtering Methodology – Step 2

- Unless filtering in the field samples must be stored, during prior to filtration, in a refrigerator.
- “Back at the camp” (or lab) eDNA is concentrated using filtration with a peristaltic pump or a suction pump
- Once filtration is complete, the filter paper can be frozen or dehydrated in vials with molecular-grade ethanol*.

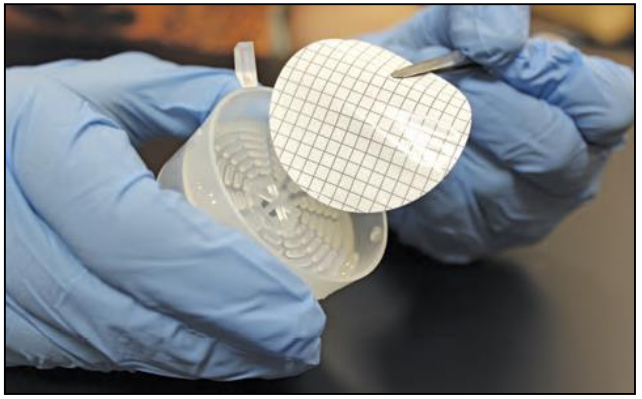
**Molecular grade ethanol is required and requires a permit to purchase – OTC ethanol will denature the DNA!*

***We’re exploring alternative methods for preservation*

Laboratory Requirements – Step 3

Hemmera works collaboratively with Dr. C. Goldberg – WSU; Caren played a lead role in the development of this new method.

- 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.

Eliminating Type 1 & Type 2 Errors

Like any field-based sampling, protocols must be followed:

- replicate samples are required to estimate occupancy while accounting for uncertainty
- Include known sites in study design to measure efficacy.
- Site sampling could include sites outside the range, or habitat tolerances, of target taxa to test potential for false positives.
- Distilled water can also be used (lab blind-test) to control for contamination during both the filtering process AND during lab-testing.
- Clean field procedures are required (different requirements at different stages depending on contamination risk)

Eliminating Type 1 & Type 2 Errors

Primer development requires a comprehensive screening process to exclude sympatric and parapatric species

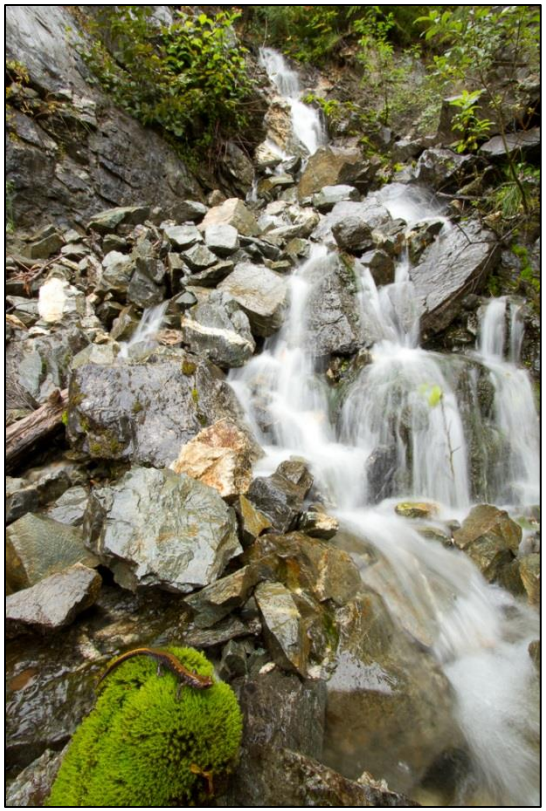
- Type 1 error: false positive detection
- Type 2 error: false negative detection

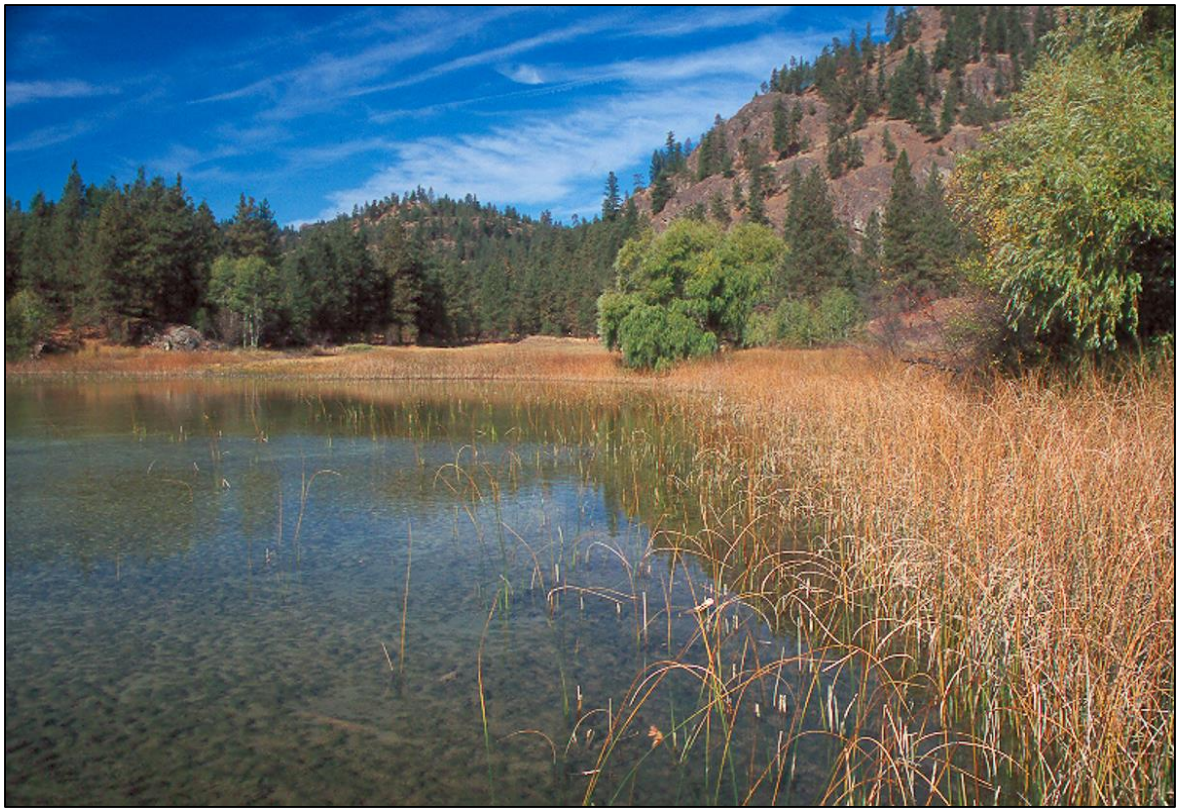
Target Taxa: Red-legged frog

Sympatric Taxa: LICL, LICA, RAPR, PSRE

Project & Survey Design Considerations

Consider sampling requirements to ensure conditions are appropriate for the system you're sampling…





Project & Survey 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???

What are the advantages of eDNA?

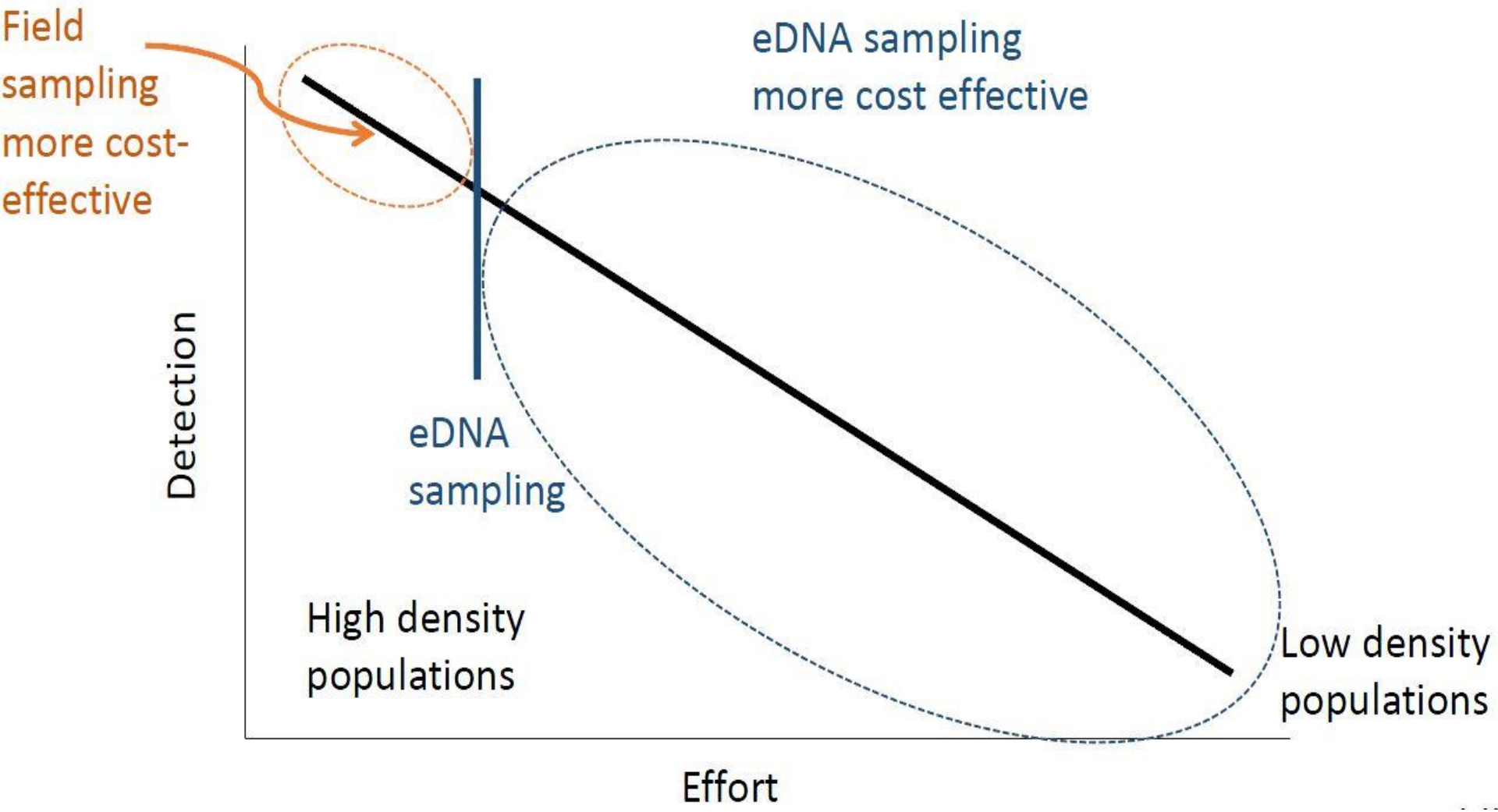
- more cost effective
- more accurate
- less invasive

eDNA: Key Strengths

- ❖ Useful for detection of inconspicuous species.
  - Detection is challenging, using conventional methods, for cryptic species that occur in low densities, have discontinuous distributions or secretive life-histories (nocturnal, low observability).

- eDNA facilitates early detection and monitoring for species of management and/or regulatory concern.

When to use eDNA: CBA



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

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, frequency and temporal proximity of use
- Precise physical proximity of target taxa (hard to define transport potential)

Reporting Results

Like any new or emerging science – perfection of methods and techniques is an evolving process based on adaptive feedback.

- “Identifying sources of error or uncertainty is a critical process in any study, especially for monitoring programs where results could influence future management decisions” (USGS. 2013)
- “eDNA detectability and concentration depend on production rates of individuals, environmental conditions, density of animals, and their residence time” (Pilliod et al. 2014).

Responsible reporting, clearly outlining limitations and describing assumptions, are required…but despite these caveats eDNA methods show great potential for inventory and monitoring aquatic species (USGS. 2014).

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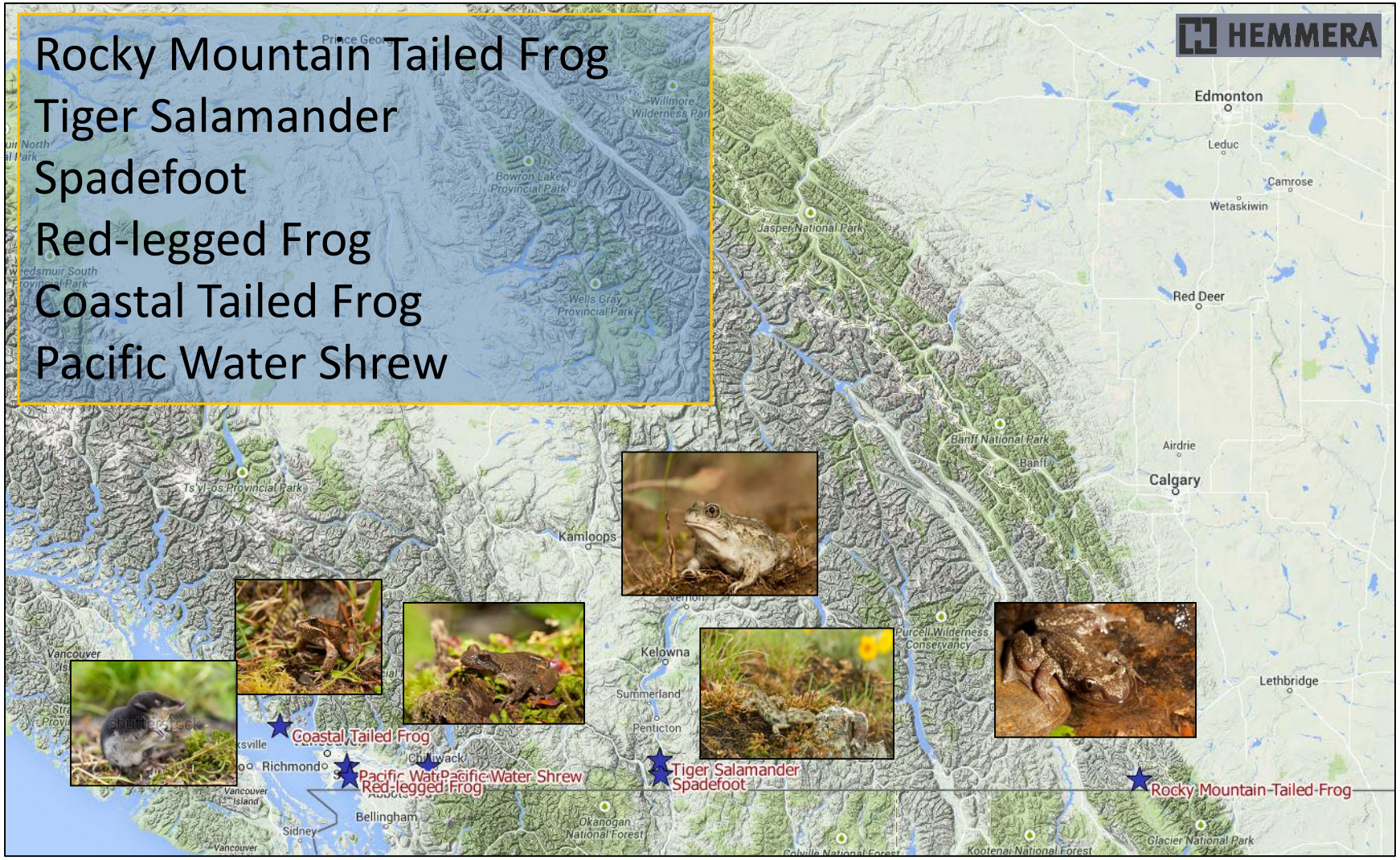
Hemmera and eDNA as a service offering

Our experiences….and we're just getting started!

Hemmera: Current BC Projects



Hemmera: Current Yukon Projects

- Western Toad
- Columbia Spotted Frog
- Chinook Salmon
- Bull Trout
- Ranavirus
- Bd

Chinook

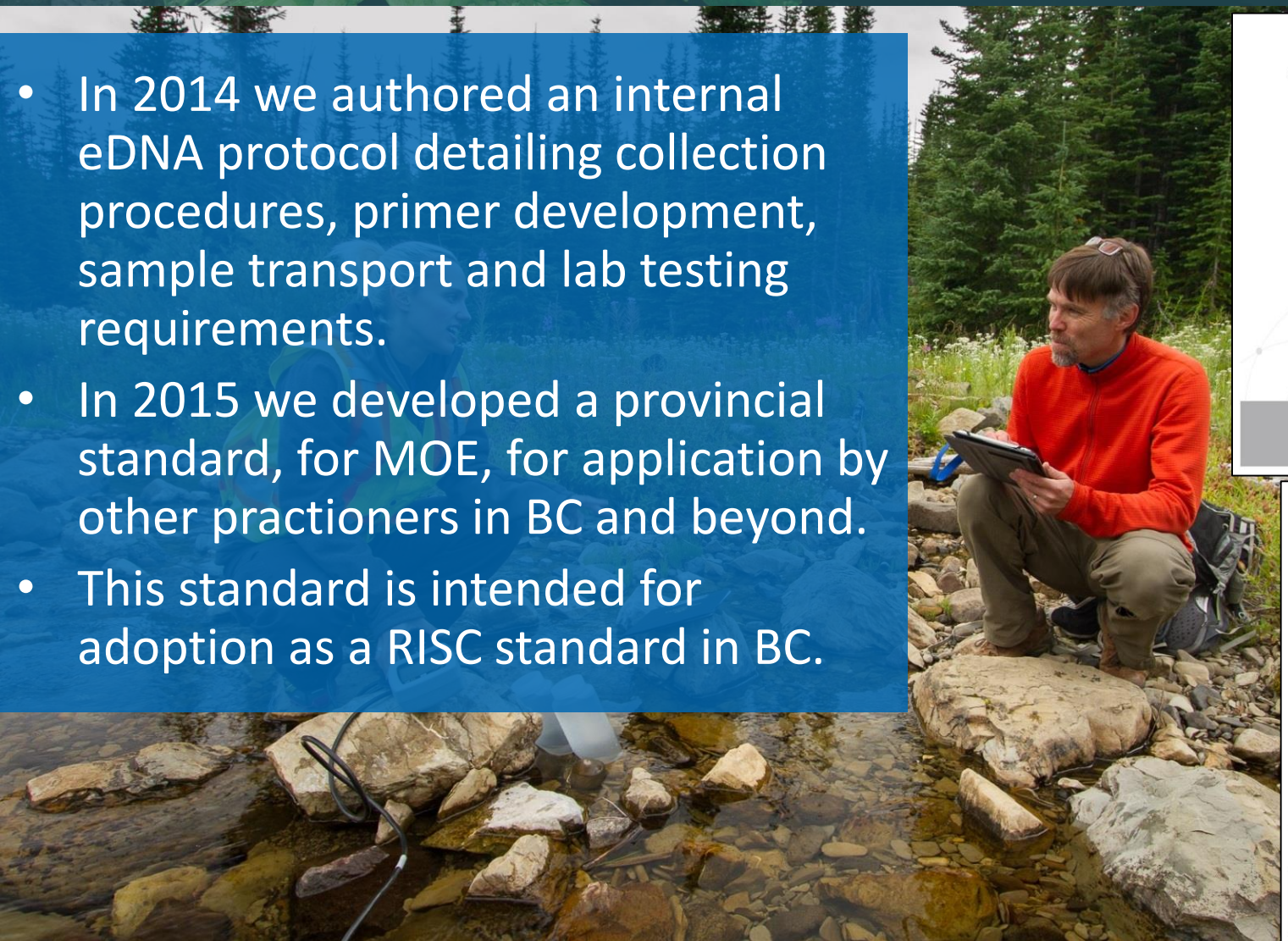
Columbia Spotted Frog

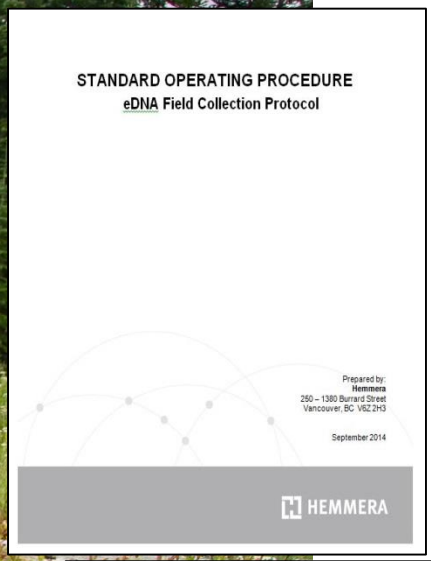
Chinook/Bull Trout

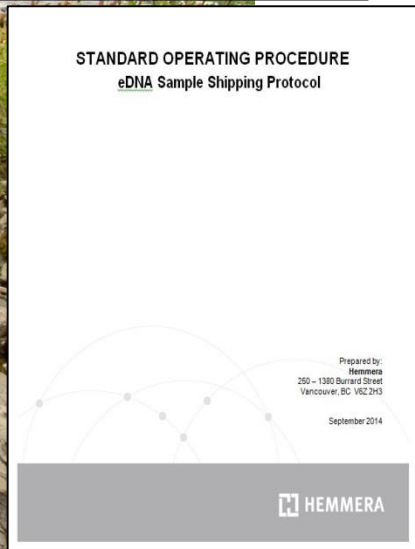
Western Toad

Standard Operating Procedures

- In 2014 we authored an internal eDNA protocol detailing collection procedures, primer development, sample transport and lab testing requirements.
- In 2015 we developed a provincial standard, for MOE, for application by other practioners in BC and beyond.
- This standard is intended for adoption as a RISC standard in BC.







Current Applications

- 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 confirming eradication of all invaders" (USGS 2012)

- eDNA facilitates early detection and monitoring (Presence/Not-detected) for management for:
  - Species of regulatory concern
  - Pathogens
  - Invasive species

Future Expanded Application

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

- Inventory for an increasing number of S@Risk
- Assess effectiveness of restoration programs
- Support environmental assessment processes
- Assess effectiveness of control programs for invasive species
- Develop monitoring programs for management purposes (relative abundance estimates of multiple sites on a temporal scale)

Thank you. Questions?

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*