DETERMINING DISTRIBUTION OF SPRING-CHINOOK USING eDNA

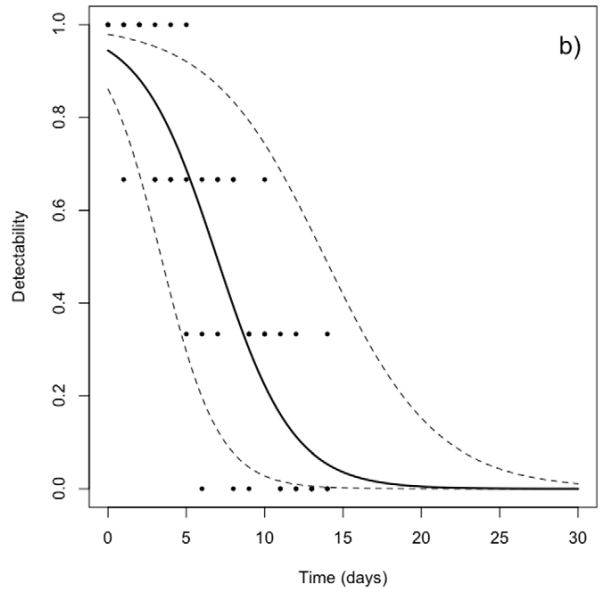
Matthew B. Laramie, US Geological Survey
Forest and Rangeland Ecosystem Sciences Center, Boise, Idaho; mlaramie@usgs.gov

Outline

- What is eDNA? And eDNA Methodology
- Project Goals, Challenges & Accomplishments
- eDNA Sample Sites
- Integrating eDNA in CJHP
- Data Sharing
- Conclusions

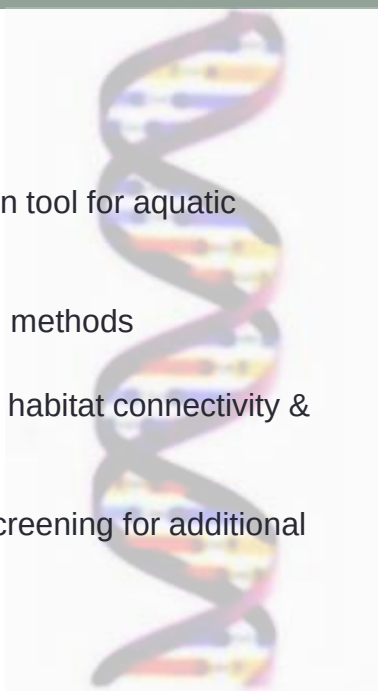
What is eDNA?

- Environmental DNA (eDNA) is dissolved or cell-bound DNA that persists in the environment
- Naturally sloughed cells, excretions, decaying tissue
- Can be collected & concentrated
- Limited persistence in freshwater
- Broken down by UV, bacterial & mechanical digestion



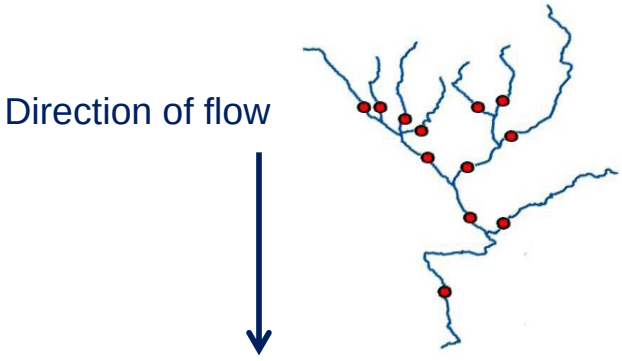
DNA detectability in freshwater according to time, under natural conditions (Dejean et al 2011)

What is eDNA?

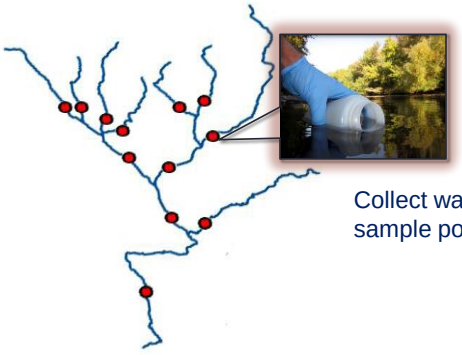


- An effective, 'sight-unseen'-detection tool for aquatic systems
- Increased sensitivity over traditional methods
- Cost-effective method of monitoring habitat connectivity & species distribution
- Archived samples allow for future screening for additional species

eDNA Methodology



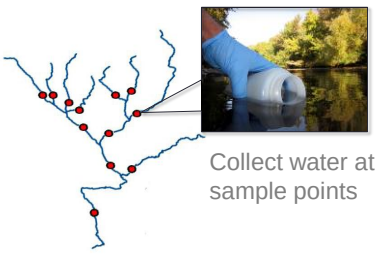
eDNA Methodology



Collect water at sample points

eDNA Methodology

1.



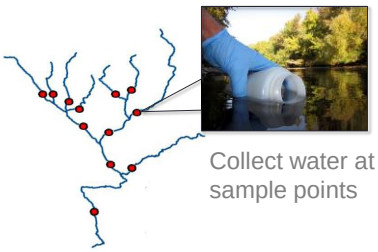
Collect water at sample points

2.

Filter water to concentrate DNA

eDNA Methodology

1.

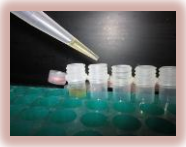


Collect water at sample points

2.

Filter water to concentrate DNA

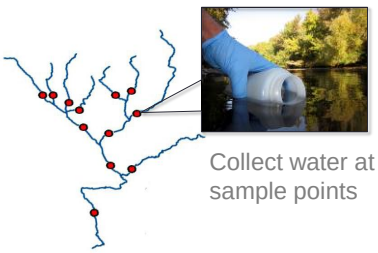
3.



Use qPCR with species-specific primers & probe

eDNA Methodology

1.

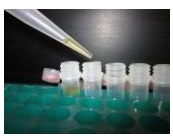


Collect water at sample points

2.

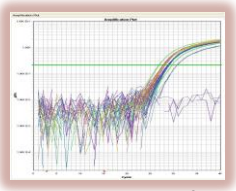
Filter water to concentrate DNA

3.



Use qPCR with species-specific primers & probe

4.



Screen PCR product for target sequence

Project Goals

1. Develop and test molecular assay to detect presence of spring-Chinook DNA in filtered water samples

Project Goals

1. Develop and test molecular assay to detect presence of spring-Chinook DNA in filtered water samples

2. Conduct eDNA sampling throughout Methow Sub-basin
   - eDNA-based distribution vs known/modeled distributions

Project Goals

1. Develop and test molecular assay to detect presence of spring-Chinook DNA in filtered water samples

2. Conduct eDNA sampling throughout Methow Sub-basin
   - eDNA-based distribution vs known/modeled distributions

3. Conduct eDNA sampling throughout Okanogan Sub-basin (US & CAN)
   - Pre-hatchery release (baseline distribution)
   - Monitor re-colonization

Project Challenges & Accomplishments

1. Design species-specific primers & probe
2. Acquire library of genetic quality fin-clips
3. Screen against target species tissue
   - Chinook (throughout geographic range)
4. Screen against non-target tissue
   - Closely related fish

Project Challenges & Accomplishments

Molecular assay

Limitations

- Assay is species-specific
- Requires temporal and/or spatial sampling considerations to target spring-Chinook

Temporal and spatial sampling

Vaseux Creek, BC CAN June 20, 2012

Temporal and spatial sampling

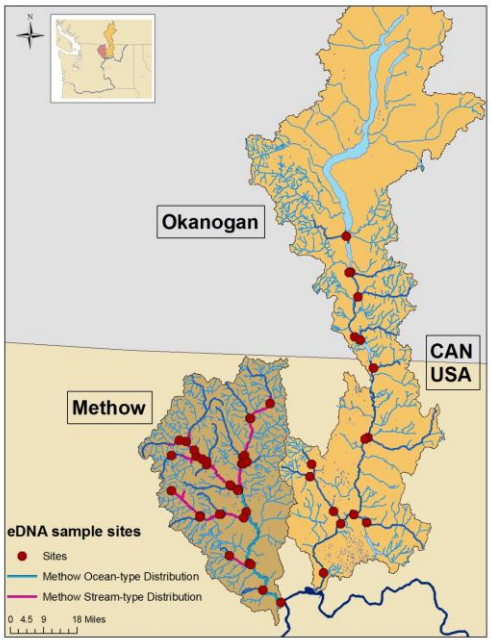
Vaseux Creek, BC CAN August 16, 2012

Temporal and spatial sampling

Salmon Creek, WA USA August 14, 2012

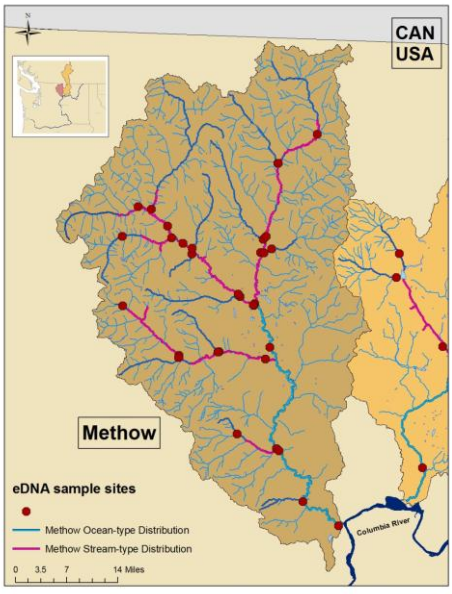
Project Goals
eDNA sampling

- 49 Sites throughout Methow & Okanogan Sub-basins



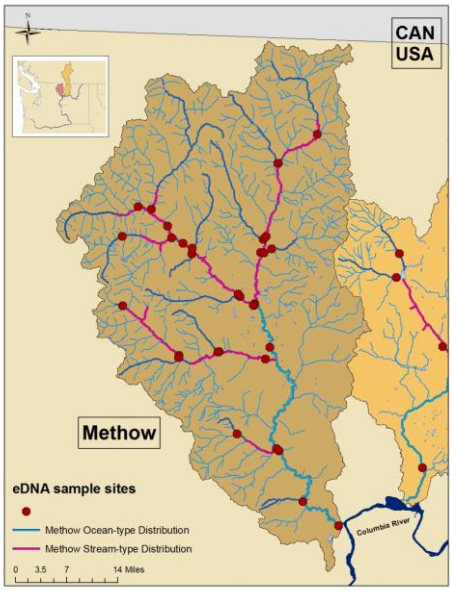
Project Goals
2) Methow

- Purpose: demonstrate effectiveness of eDNA detection of spring-Chinook in large UCR watershed
- Sampling completed in 2012
- Analysis in progress (expected completion Spring 2013)



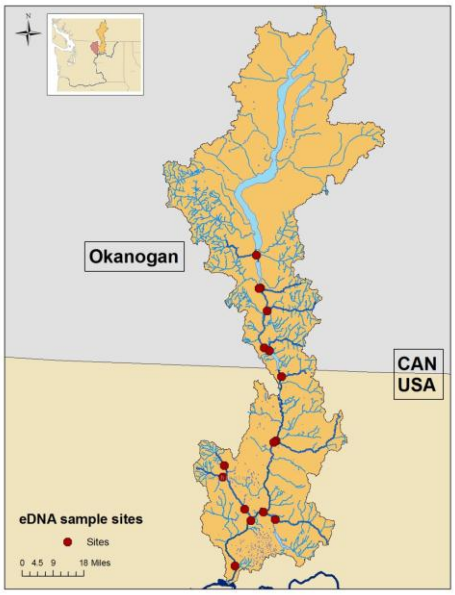
Project Goals
2) Methow

- 33 sites
- 2 sampling events
  - June-August 2012
- 3 1-L water samples per site x event = 198 total samples
- H2O temperature (C)
- GPS coordinates (UTM)



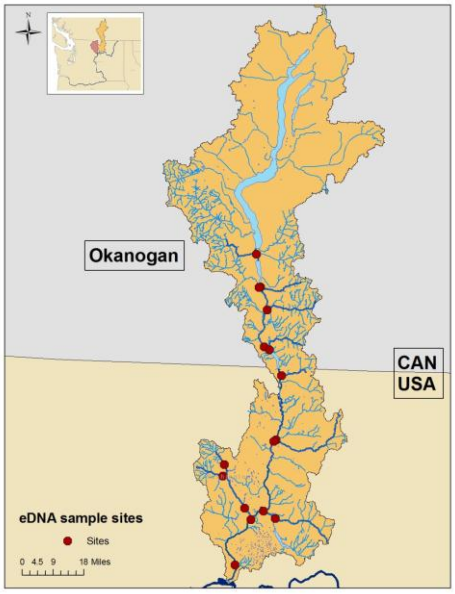
Project Goals
3) Okanogan

- Purpose: Baseline survey of spring-Chinook presence in Okanogan Sub-basin
  - pre-hatchery release
- Sampling completed in 2012
- Analysis in progress (expected completion Spring 2013)



Project Goals
3) Okanogan

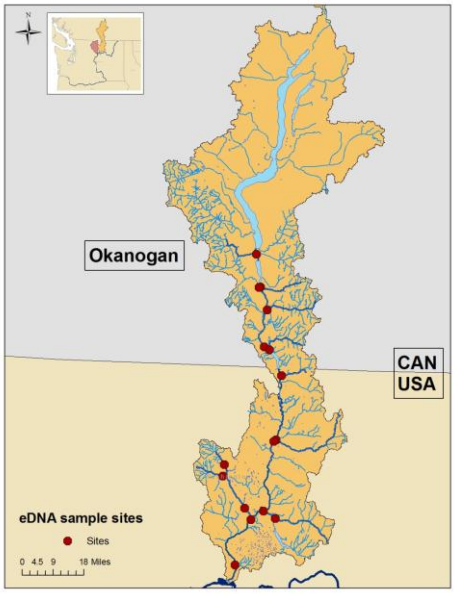
- 16 Sites
- 2 sampling events
  - June-August, 2012
- 3 samples per site x event = 96 total samples
- H2O temperature (C)
- GPS coordinates (UTM)



Project Goals
3) Okanogan

<u>Focus</u>

- Potential for spring-Chinook re-colonization
  - Barriers
  - Habitat requirements
- Accessibility
  - Road crossings
  - Turnouts

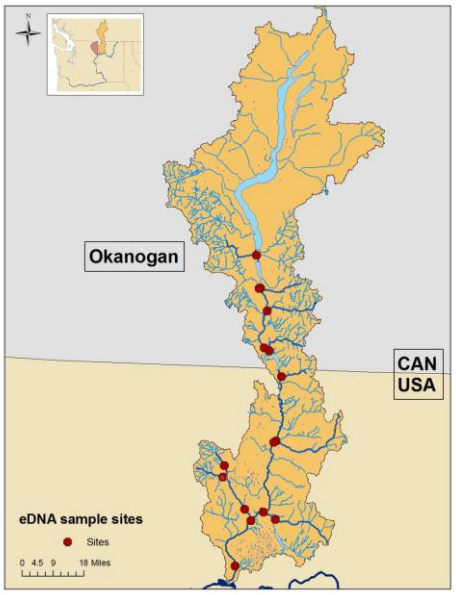


Site accessibility

Sites easily accessible (road crossings, turnouts)
Salmon Creek, WA USA

Project Goals
3) Okanogan

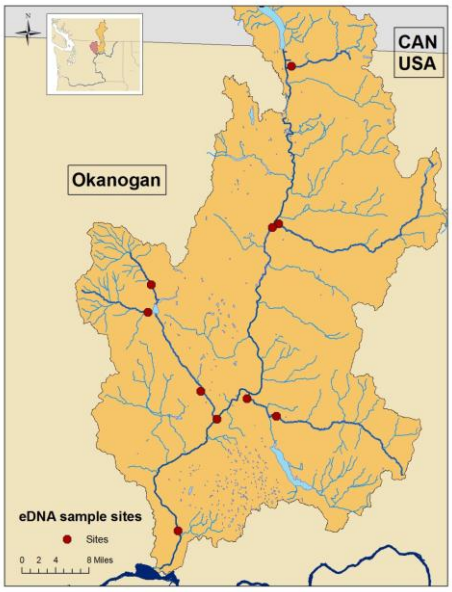
- Hyp: Rapid & cost-effective
  - Prioritize resources & efforts
  - Monitoring



Project Goals
3) Okanogan

USA

- Okanogan River
- Salmon Creek
- North Fork Salmon Creek
- West Fork Salmon Creek
- Omak Creek
- Bonaparte Creek
- Nine Mile Creek

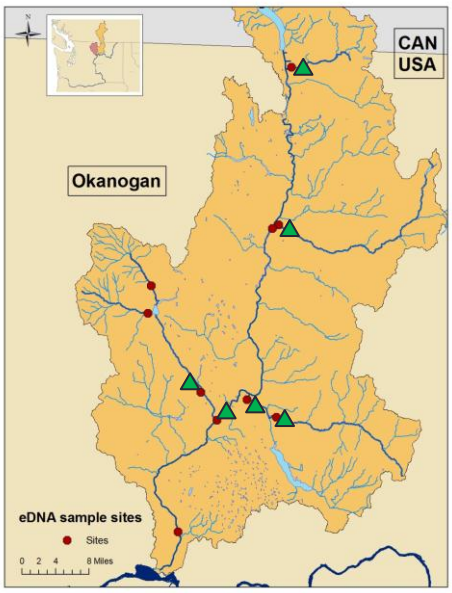


Project Goals
3) Okanogan

USA

- Okanogan River
- Salmon Creek
- North Fork Salmon Creek
- West Fork Salmon Creek
- Omak Creek
- Bonaparte Creek
- Nine Mile Creek

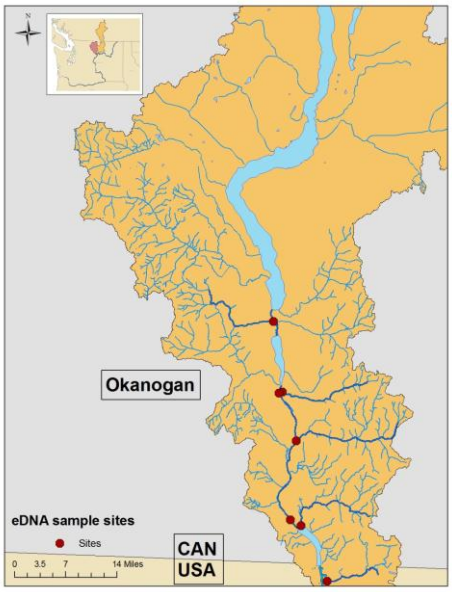
▲ = OBMEP ANNUAL SITE



Project Goals
3) Okanogan

CANADA

- Inkaneep Creek
- Okanogan River
- Vaseaux Creek
- Shuttleworth Creek
- Shingle Creek

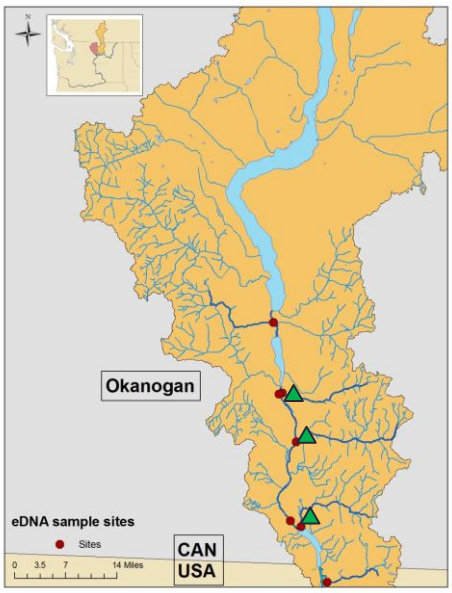


Project Goals
3) Okanogan

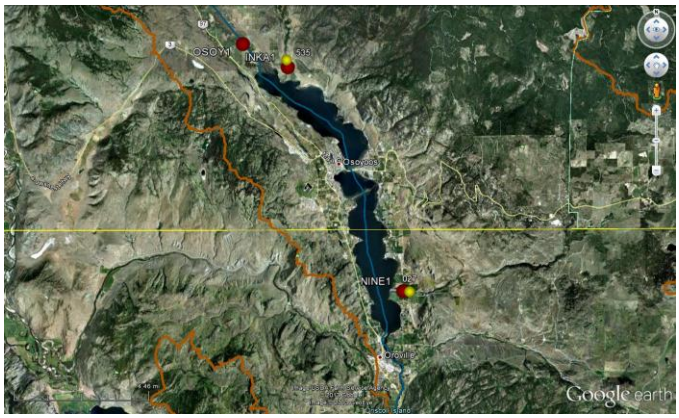
CANADA

- Inkaneep Creek
- Okanogan River
- Vaseaux Creek
- Shuttleworth Creek
- Shingle Creek

▲ = OBMEP ANNUAL SITE



eDNA Sites



NINE1 – Nine Mile Creek, at CCT pit-tag site
INKA1 – Inkaneep Creek, at rd crossing
OSOY1 – Okanogan River, above Lake Osoyoos

Appropriate uses of eDNA

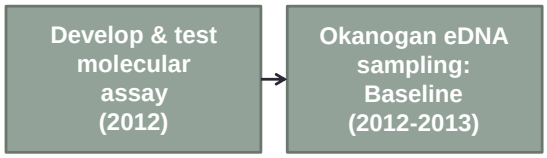
- Presence/absence data (esp. at low density)
- When efficiency of traditional methods reduced
  - Periods of high stream flow
  - Difficult/unsafe to electro-fish or snorkle
  - Weir traps compromised
- Relationship: species density vs required survey effort
  - Low-density → high-effort required to detect
  - High-density → low-effort required to detect
    - eDNA could be useful when density is low, as alternative to investing high-effort

Integrating eDNA sampling to monitor CJHP spring-Chinook re-establishment

eDNA integration into CJHP

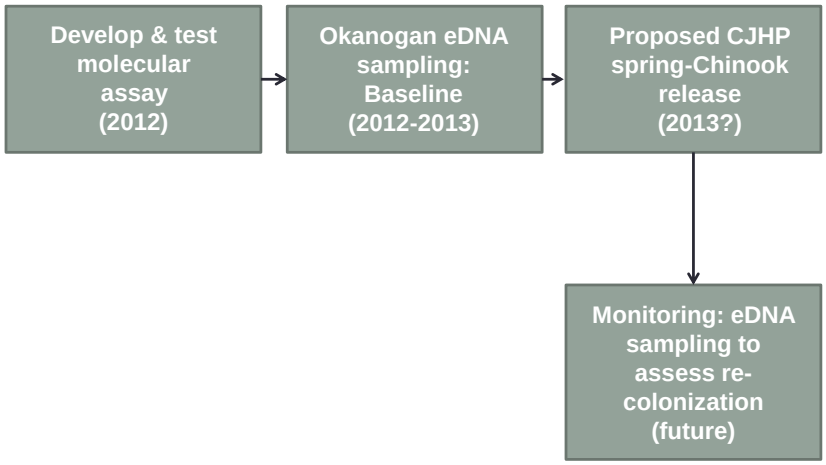
Develop & test molecular assay (2012)

eDNA integration into CJHP

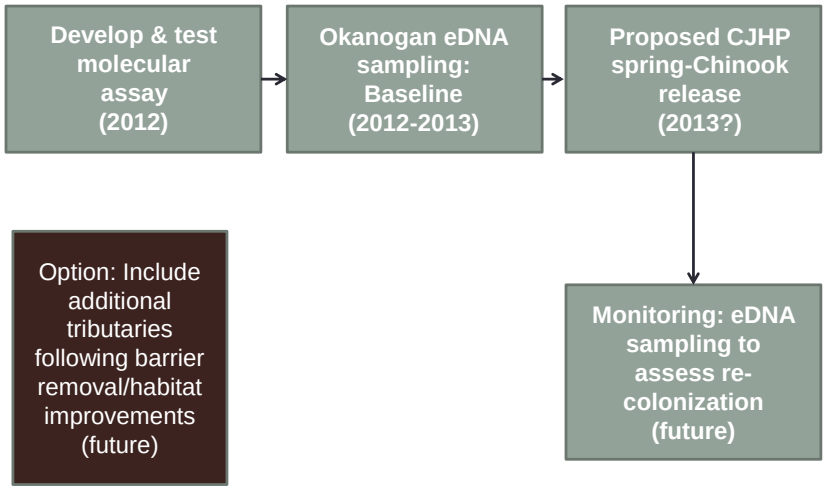


eDNA integration into CJHP

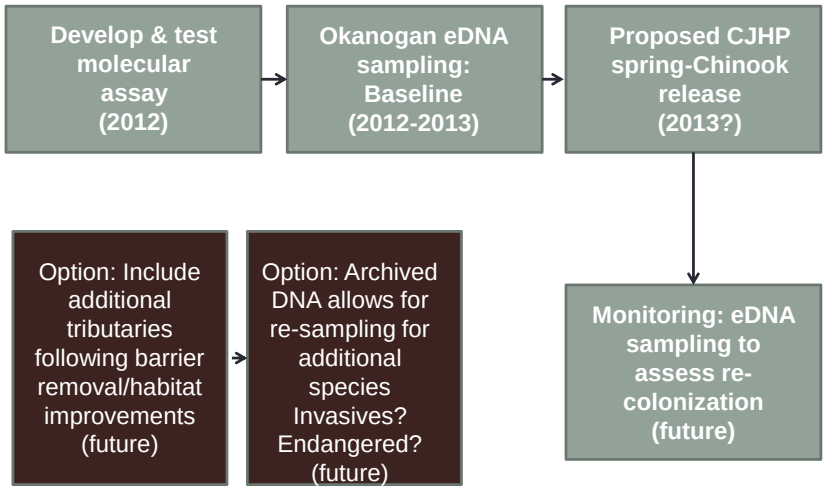
eDNA integration into CJHP



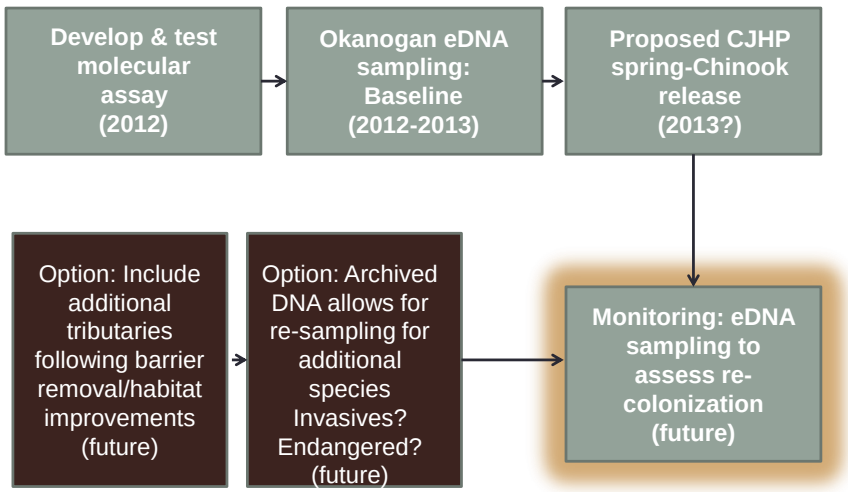
eDNA integration into CJHP



eDNA integration into CJHP



eDNA integration into CJHP



Collaborations

- Sample site coordination
  - OBMEP and WDFW
  - Re-colonization potential
  - Data gaps
  - Ease of access
- Aligned eDNA sites with OBMEP sites
  - Habitat & Snorkel sites (Annual and Panel)
- Coordinated with CJHP planning
  - Surveyed prior to proposed hatchery release

Data Management/Data Sharing

- M.S. Thesis (BSU) – Summer 2013
- Peer-reviewed publications – 2013+
- Conference presentations
  - BOBTWG Annual Meeting - Penticton, BC March 1, 2013
  - CJHP Annual Review – Bridgeport, WA. March 2013
  - AFS Western Regional Conference - Boise, ID. April, 2013
  - ICCB Annual Conference – Baltimore, MD. July, 2013

Data Management/Data Sharing

- Annual reports
- Direct access to data (GNLCC DMP)
- Archived DNA samples
  - Stored at -20C (BSU)

USGS Grant Proposal: TESNAR - Technical Training in Support of Native American Relations

- Course Title: Applying environmental DNA (eDNA) methods for improving species detection when sampling freshwater rivers and streams
- 1 Day workshop (class & in-field)

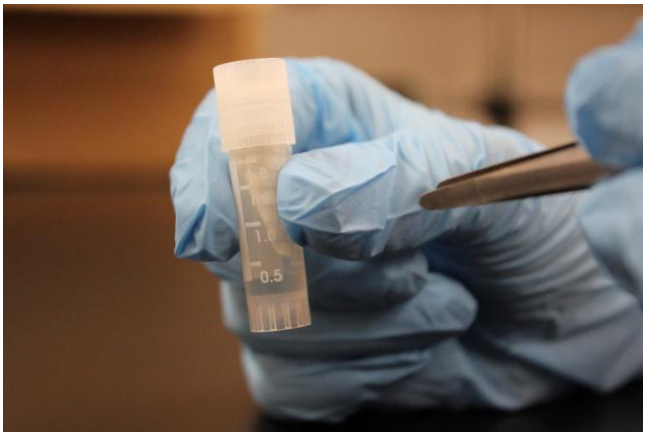
Conclusions

- eDNA may be a sensitive method for detection of spring-Chinook

Conclusions

- Could allow for rapid, cost-effective surveys
  - Presence/absence
  - Re-colonization
  - Prioritize resources

Conclusions



Archived samples allow for additional species detection

SUPPLEMENTAL MATERIAL

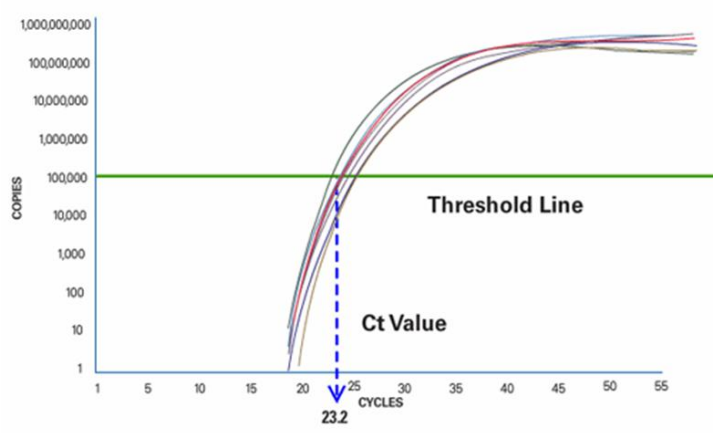
Inference with eDNA detection

Gradient of evidence for species presence

Strength of evidence	eDNA observations
Stronger	Repeated trips with positive samples over different years
	Repeated trips with positive samples within a year
	Multiple positive samples from single trip
Weaker	Single positive samples

(Jerde et al 2011)

Quantitative PCR (qPCR)



(www.appliedbiosystems.com)

Cytochrome oxidase subunit I (COI)

- mtDNA - Mitochondrial
  - higher copy number
- Relatively conserved region
  - Low variation within species
  - Suitable variation between species
- Many sequences available (GenBank)
  - 232 chinook sequences available
    - Sampled throughout range

(Rasmussen et al 2010, Rasmussen et al 2009)

Advantages of qPCR for eDNA

- Reduced potential for false positive results

(Thomsen et al 2011)

Advantages of qPCR for eDNA

- Reduced potential for false positive results

(Thomsen et al 2011)

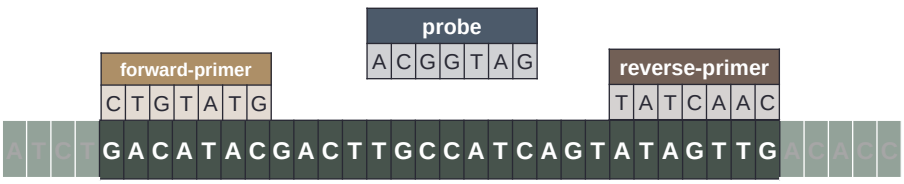
Advantages of qPCR for eDNA

- Reduced potential for false positive results

(Thomsen et al 2011)

Advantages of qPCR for eDNA

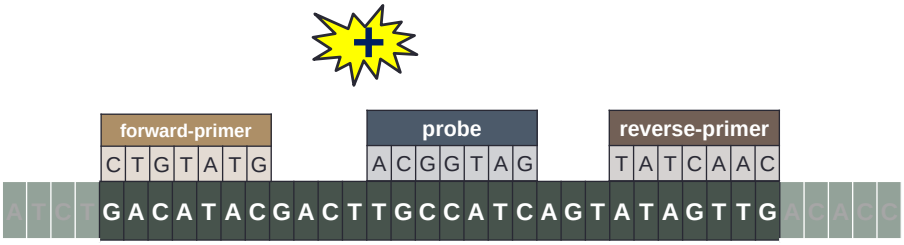
- Reduced potential for false positive results



(Thomsen et al 2011)

Advantages of qPCR for eDNA

- Reduced potential for false positive results



(Thomsen et al 2011)

Advantages of qPCR for eDNA

- Reduced potential for false positive results
- No post-PCR processing
- Turn around time, sample throughput

(Thomsen et al 2011)

Studies using eDNA methods

- American Bullfrog in lentic environments
  (Ficetola et al 2008)

Studies using eDNA methods

- Asian Carp in large canal systems
  (Jerde et al 2011)

Studies using eDNA methods

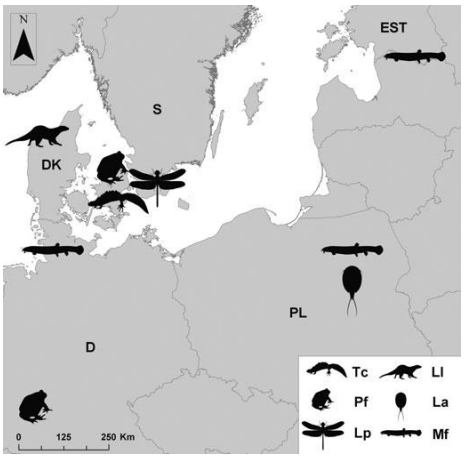
- Rocky Mountain Tailed-frog & Idaho Giant Salamander in headwater streams

  (Goldberg, C. , Pilliod, D., Arkle, R. , Waits, L., 2011)

Studies using eDNA methods

- Spadefoot toads
- Great Crested Newts
- weather loach
- dragonflies
- Eurasian otter
- Fairy shrimp

  (Thomsen et al 2011)



Monitoring endangered freshwater biodiversity using environmental DNA

eDNA Sites

(OK1) Okanogan River - Gauging station above Monse, WA

eDNA Sites

SAL1 – Salmon Creek, at Spring Creek Rd.
OK2 – Okanogan River, above Salmon Creek confluence

eDNA Sites

WFSAL1 – West Fork Salmon Creek, at 'State Lands' sign
NFSAL1 – North Fork Salmon Creek, at Cottonwood CG

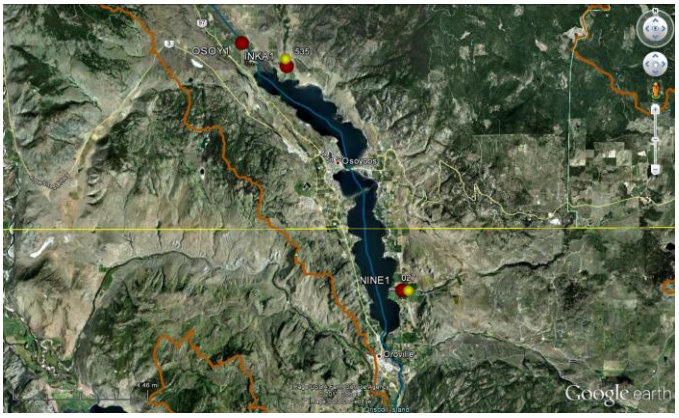
eDNA Sites

OMAK1 – Omak Creek at turnout by mill
OMAK2 – Omak Creek above Mission Falls

eDNA Sites

OK3 – Okanogan River, below Bonaparte Creek
BONA1 – Bonaparte Creek, at Bretz Rd.

eDNA Sites



NINE1 – Nine Mile Creek, at CCT pit-tag site
INKA1 – Inkaneep Creek, at rd crossing
OSOY1 – Okanogan River, above Lake Osoyoos

eDNA Sites

VAS1 – Vaseux Creek, at HWY 97

eDNA Sites

SKAHA1 – Okanogan River, below Skaha Lake
SHUT1 – Shuttleworth Creek, at HWY 97
SHING1 – Shingle Creek, at eCommunity Building