USING eDNA ANALYSIS TO DETERMINE THE PRESENCE OF AQUATIC SPECIES

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Western Forestry and Conservation Association
5th Field Technologies for Data Collection in Forestry, Fisheries, and Natural Resources
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Outline

- Overview
- Discerning salmon redds using eDNA
- eDNA as an index of fish abundance
- Protocols
- Resources
Aquatic survey methods
Aquatic survey methods

Electrofishing

Snorkeling
Aquatic survey methods

Electrofishing

Snorkeling
eDNA
The Basic Approach

1. Collect Water Sample
2. Concentrate DNA
3. Extract DNA (all) and Amplify Target Sequence
4. Screen for DNA Presence (Infer Species Presence)
[eDNA] = production - degradation

Example: fish

eDNA production
- fish density
- fish health
- reproductive status
- metabolism

eDNA degradation
- UVB exposure
- water temperature
- adsorption
- pH

Influence

Environment
- water volume
- water temperature
- habitat
How long does DNA persist in water?

1, 5, 10 bullfrog tadpoles in 900 mL beaker for 5 days

1 sturgeon in 3 ponds (12 m²) for 10 days

Where should samples be collected?

Chinook

Hatchery

## Integrating into Existing Monitoring Programs

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*Highly sensitive eDNA methods could be a useful alternative to investing high effort.*
Integrating into Existing Monitoring Programs

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eDNA survey methods

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Monitoring Salmon Populations

Photo used with permission; © Brian Miller (CCT/OBMEP)
Monitoring Salmon Populations

Chinook Redds

Cedar River, Washington Department of Fish and Wildlife
Habitat & timing used to differentiate redds where species co-occur
1. How much salmon DNA is in the environment (water column and gravel) during spawning?

2. Can we differentiate coho redds from chinook redds using eDNA analysis?
1. 15 mL water samples (triplicate)
2. Field preserved with 1.5 mL sodium acetate and 33 mL ethanol
3. DNA extracted via precipitation method (Ficetola et al. 2008)
4. qPCR analysis
How much Coho DNA is at a Coho Redd?
Can unknown reds be assigned?

Coho DNA (pg/μL + 1)

Redds of unknown origin from Still Creek (Tributary of Zigzag River)

- Known Coho Redds
- Unknown Redds
Can unknown redds be assigned?

Still Creek (Tributary of Zigzag River)

Cohu DNA (pg/μL + 1)

A

B

C

Water

Unknown Redds
All streams combined

(a) Coho eDNA

(b) Chinook eDNA
eDNA concentration as an index of fish abundance?
Omak Creek

- Mid-size perennial stream
- ~5 m wetted width
- 10 - 150 cfs
- USGS Gage 12445900
1. Does [eDNA] reflect relative fish abundance?
2. Does it matter where samples are collected (cross-section)?

Reach length eDNA (diff.); \( r_i = \overline{d}_i - \overline{u}_i \)

- >95mm: 5,918 ± 607 (95% CI)
- <95mm: 18,626 ± 1,953
- TOTAL: ~ 24,500 RBT

Total stream length ~ 9km
Electrofish mark-recap RBT abundance

Reach RBT Density (fish/150m)

Electrofish M-R abundance

Fish size

RBT abundance (fish/150m)

Reach

Does [eDNA] reflect fish abundance?

RBT eDNA (reach length diff.) vs. RBT abundance (fish/150m)

\[ R^2 = 0.5912 \]
eDNA as an index of relative abundance
eDNA as an index of relative abundance
eDNA within the stream cross-section
eDNA Sampling Protocols

http://pubs.usgs.gov/tm/02/a13/tm2a13.pdf
Selecting the best protocol

- Are you sampling in remote locations, away from an electrical power source (120-V AC outlet, vehicle battery)?
  - YES
    - Is the weight of sampling equipment a primary concern?
      - YES
        - Use Protocol #1: Manual hand pump
      - NO
        - Use Protocol #2: Pump head with rechargeable cordless driver/drill
  - NO
    - Use Protocol #3: 120-V AC peristaltic pump
Step 1: Choose the best protocol, depending on your conditions

Protocol #1: Hand pump
Protocol #2: Cordless driver
Protocol #3: 120-V pump

Step 2: Collect water sample

Direct
Collect and pour

Step 3: Preserve water sample

Ethanol-filled vial
Protocol #1: Hand pump
Protocol #2: Cordless driver
Protocol #3: 120v pump
Sample collection options
Comparing sample collection options

Pilliod et al. (2013). Canadian Journal Fisheries and Aquatic Sciences.

\[ P = 0.02 \text{ Instream samples higher} \]
Sample preservation

Filters stored in ethanol at room temp
eDNA Resources

**SAMPLING PROTOCOLS**

http://pubs.usgs.gov/tm/02/a13/tm2a13.pdf

**USGS FACT SHEET**

eDNA Resources

eDNA.fisheries.org
Acknowledgements

• Portland Water Bureau (PWB)
  Burke Strobel

• Colville Confederated Tribes (CCT)
  Okanogan Basin Monitoring & Evaluation Program (OBMEP)
  Chief Joseph Hatchery Science Program (CJHP)

• Washington State University (WSU)
  Caren Goldberg & Kath Strickler
What does an eDNA sample represent?

Pilliod et al. (2013) Canadian Journal of Fisheries and Aquatic Sciences 70:1123-1130.
(a) Coho eDNA

(b) Chinook eDNA
### Table 3. Analysis of Variance (ANOVA) table for differences in O. kisutch eDNA among sample types.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample type</td>
<td>6</td>
<td>1847497</td>
<td>307916</td>
<td>8.1409</td>
<td>1.997e-06 ***</td>
</tr>
<tr>
<td>Residuals</td>
<td>59</td>
<td>2231569</td>
<td>37823</td>
<td></td>
<td></td>
</tr>
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</table>

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Response: Site replicate mean [eDNA] (pg/15 mL)

### Table 4. Tukey multiple comparisons of means w/ 95% family-wise confidence level for O. tshawytscha eDNA among sample types.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>diff</th>
<th>lwr</th>
<th>upr</th>
<th>p adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. tshawytscha redd - gravel</td>
<td>266.7449</td>
<td>120.5793</td>
<td>412.91037</td>
<td>0.0000133</td>
</tr>
<tr>
<td>O. tshawytscha redd - O. kisutch redd</td>
<td>291.8099</td>
<td>145.6444</td>
<td>437.97538</td>
<td>0.0000018</td>
</tr>
<tr>
<td>Water - O. tshawytscha redd</td>
<td>-269.2079</td>
<td>-387.1876</td>
<td>-151.22823</td>
<td>0.0000001</td>
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Fit: aov(formula = Site replicate mean [eDNA] (pg/15mL)~ Sample type, data = O. tshawytscha eDNA)
### Possible detection outcomes at a site

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<th>Outcomes</th>
<th>% of sites</th>
<th># replicates (-)</th>
<th># replicates total</th>
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<td>41%</td>
<td></td>
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<td>1 0 0</td>
<td>3%</td>
<td>6</td>
<td>9</td>
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<tr>
<td>1 1 0</td>
<td>7%</td>
<td>6</td>
<td>18</td>
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<td>1 1 1</td>
<td>49%</td>
<td>0</td>
<td>135</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>12</td>
<td>162</td>
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Laramie, M.B. (2013) [http://scholarworks.boisestate.edu/td/780](http://scholarworks.boisestate.edu/td/780)
## Assessing detection probability and error

Laramie, M.B. (2013) [http://scholarworks.boisestate.edu/td/780](http://scholarworks.boisestate.edu/td/780)

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12/162 = 7% false negatives
Contamination Prevention

Contamination can result from various factors at every step in the sample collection process. Be vigilant. Before initiating eDNA sample collection, the following field and laboratory practices should be reviewed to avoid contamination of samples and cross-contamination among samples:

- Wear clean, non-powdered, single-use gloves when collecting samples and removing filters. Do not let gloves contact contaminated surfaces, such as any equipment that was not sterilized between sites, prior to handling the filter.
When do we use eDNA?

- High density populations
- Low density populations
- Detection
- Effort

Field sampling more cost-effective

EcoDNA sampling more cost effective

Figure courtesy of Dr. Caren Goldberg, WSU
How much Coho DNA is in environment?

Coho DNA (pg/μL + 1)
How much Coho DNA is at a Coho Redd?