

# **Identification of Steelhead and Coho Salmon Redds Through Environmental DNA Analysis**

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Salmon Partnership**

Problem: Salmon and Steelhead build redds in same time and place

- Difficult to distinguish differences between the redds of the species
  - Overlap in spawning (spatial/temporal)
- Ways to determine species of redd
  - Can identify through redd morphology and timing
  - eDNA tool can test visual identification

**Coho**

S	O	N	D	J	F	M	A	M	J	J	A
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**Steelhead**

S	O	N	D	J	F	M	A	M	J	J	A
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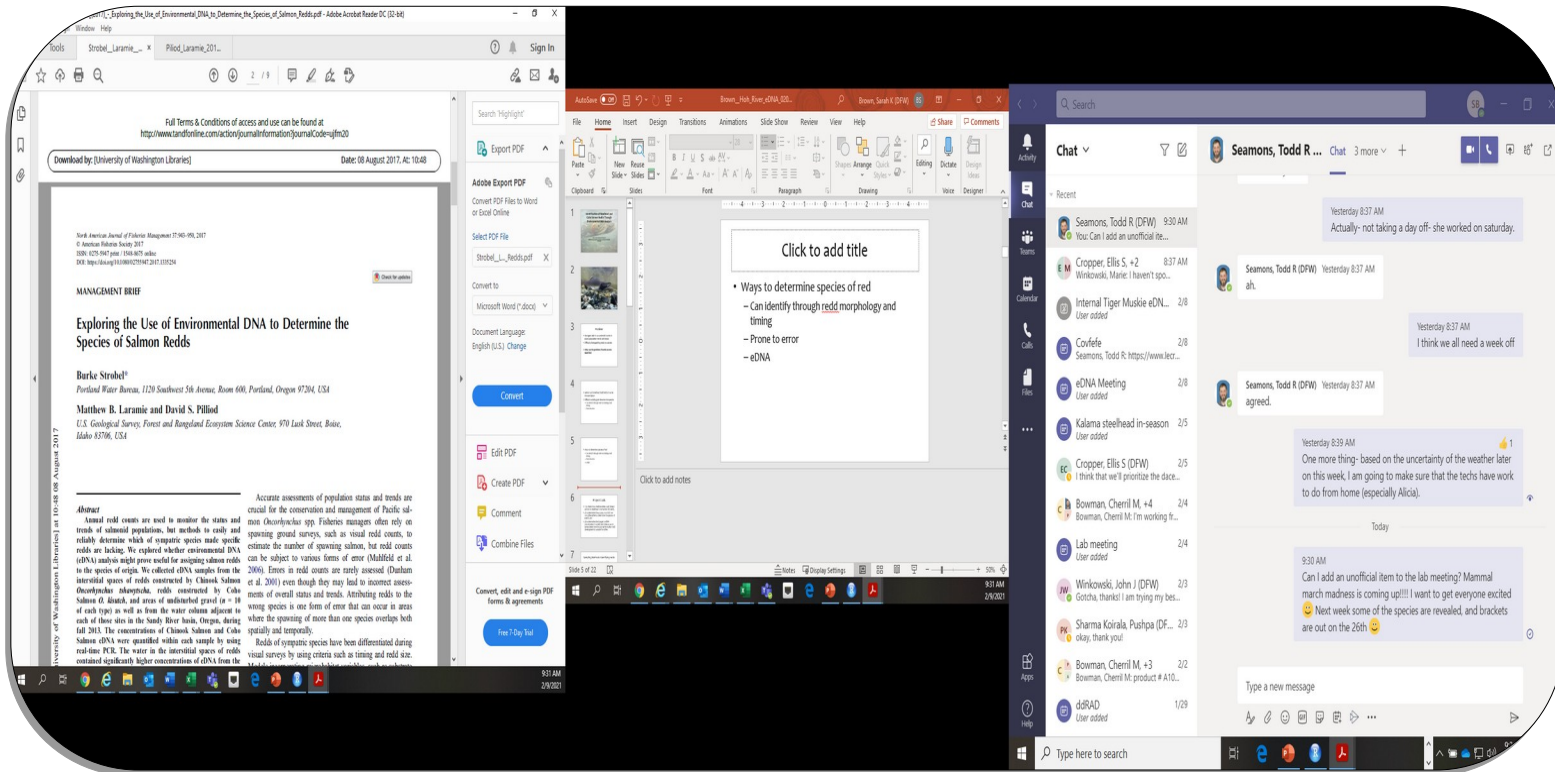
**Environmental DNA:** DNA that an organism released to the environment (water, soil, air)



### **DNA Sources**

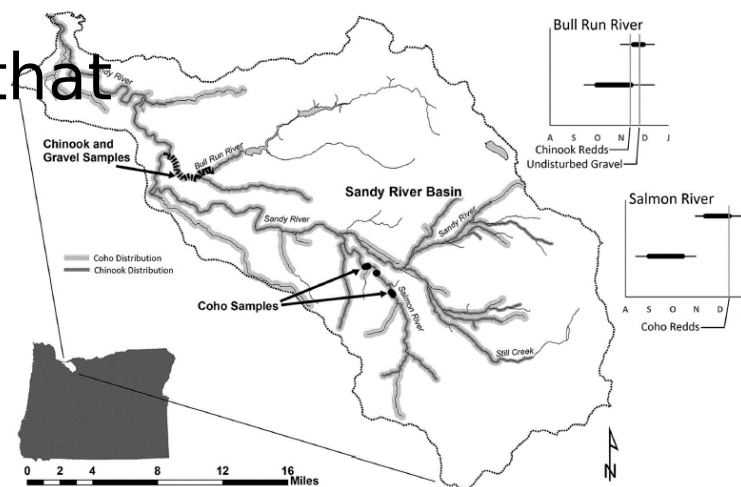
- Sloughed cells
- Mucus
- Gametes
- Urine
- Fecal matter
- Shed hair
- Carcasses





Higher Concentration of the species that made redd in:

- Redd compared to water column
- Than other species



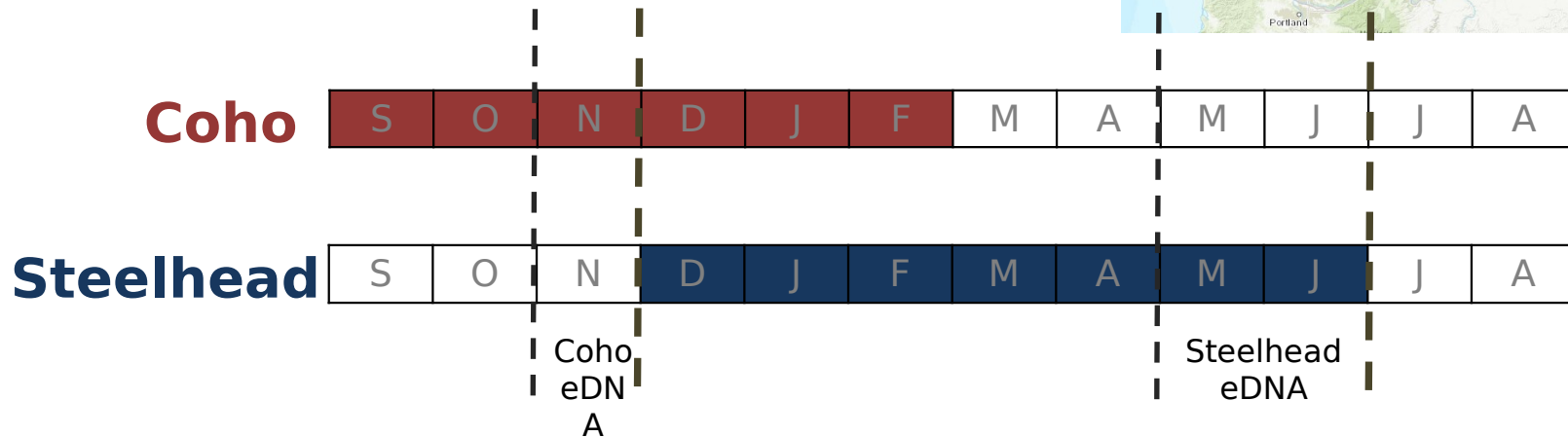
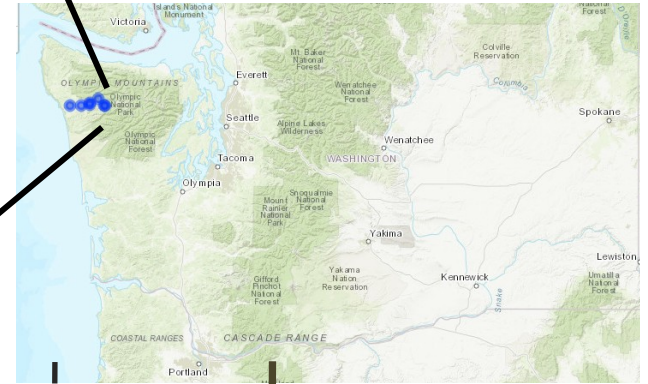
# Project Goals



Hoh River as study system

1. eDNA use for species identification of steelhead vs. coho redds?
  - a. Single eDNA sample
2. Determine change in eDNA concentration overtime?
  - a. Repeated eDNA sample

# Sampling Methods: Location





# Sampling Methods: Locations

- Lower Owl Creek (LOW)
  - Upper Owl Creek (UOW)
  - South Fork Hoh 0486 side channel (4SC)
  - Ranger Station to Twin Creek (Hoh River) (RTW)
  - Upper Big Flat (South Fork Hoh) (UBF)
  - Upper Winfield Creek (UWI)
  - Upper Lacey Seep (ULS)
- 
- 21 Steelhead redds (single sample)
  - 6 Steelhead redds (sampled every 3-4 days to detect [eDNA] change)
  - Total of 27
  - 15 Coho Redds

# Sampling Methods: Select a redd



Mara Zimmerman and John Winkowski

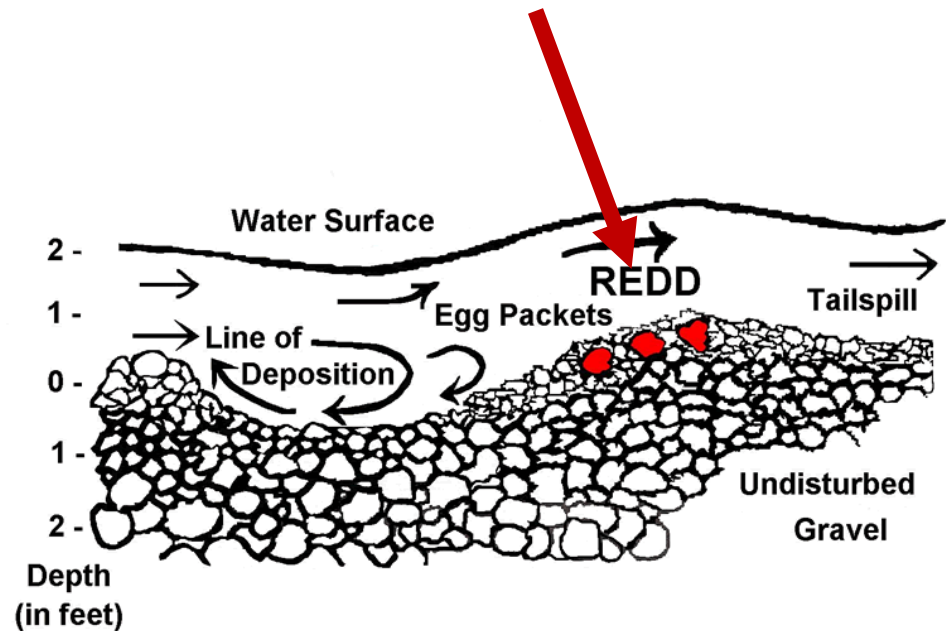
- Sampling in months to minimize overlap
- Surveyed reaches at 3-4 day intervals
- Sampling after coho/steelhead were observed constructing redd
- Field assigned species considered accurate



# Sampling Methods: Collecting 90 ml from redds

## Water Samples:

- Interstitial (from redd gravel)
- Adjacent water column
- Sterile H<sub>2</sub>O (control)



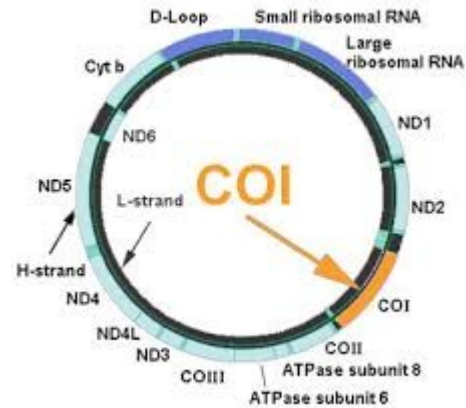
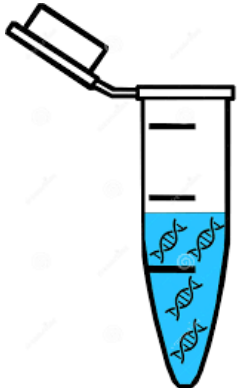
# Sampling Methods: Filtering water



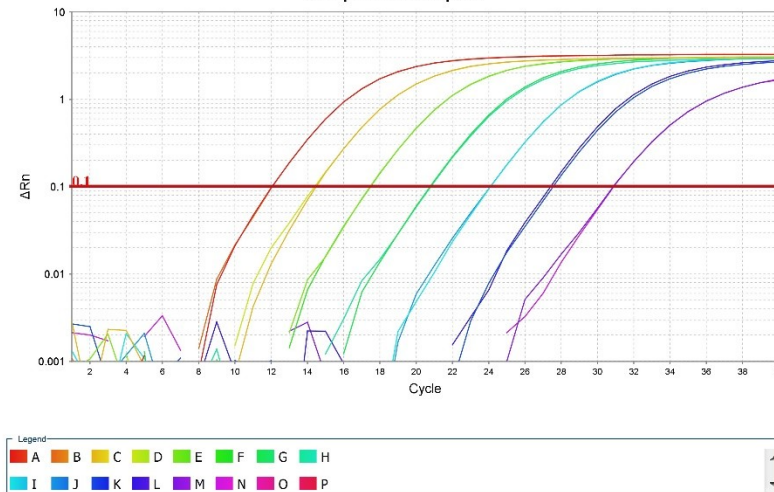
Lauren Bauernschmidt and David Low



# Sampling Methods: Laboratory Analysis



Amplification plot





1) eDNA use for species detection of redds in Hoh River tributaries?

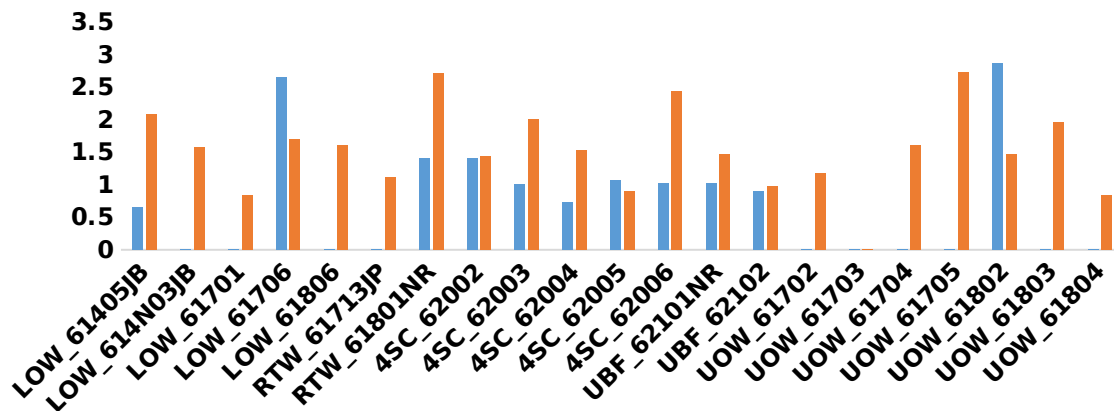
eDNA is detected in redds

	<b>Steelhead Detected</b>	<b>Coho Detected</b>	<b>Total</b>
<b>Steelhead Redd</b>	<b>16</b>	<b>4</b>	<b>20/21</b>
<b>Coho Redd</b>	<b>4</b>	<b>8</b>	<b>12/15</b>

1) eDNA use for species detection of redds in Hoh River tributaries?

## Steelhead redd samples: eDNA concentration useful (but not perfect) for redd identification

Steelhead Redds (field obs.)



### Steelhead Redds

■ Coho Log Transformed

■ Steelhead Log Transformed

Success

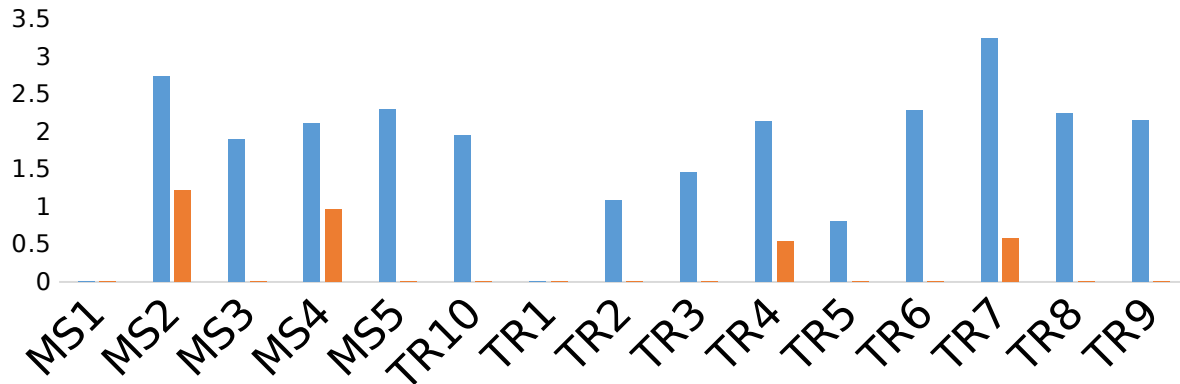
Problematic

Steelhead only	9/21
Steelhead >Coho	6/21
Coho >Steelhead	3/21
Equal	2/21
No detection	1/21

1) eDNA use for species detection of redds in Hoh River tributaries?

## Coho redd samples: eDNA concentration useful (but not perfect) for redd identification

Coho Redds (Field Obs.)



Coho Redds

■ Coho Log Transformed

■ Steelhead Log Transformed

Success {

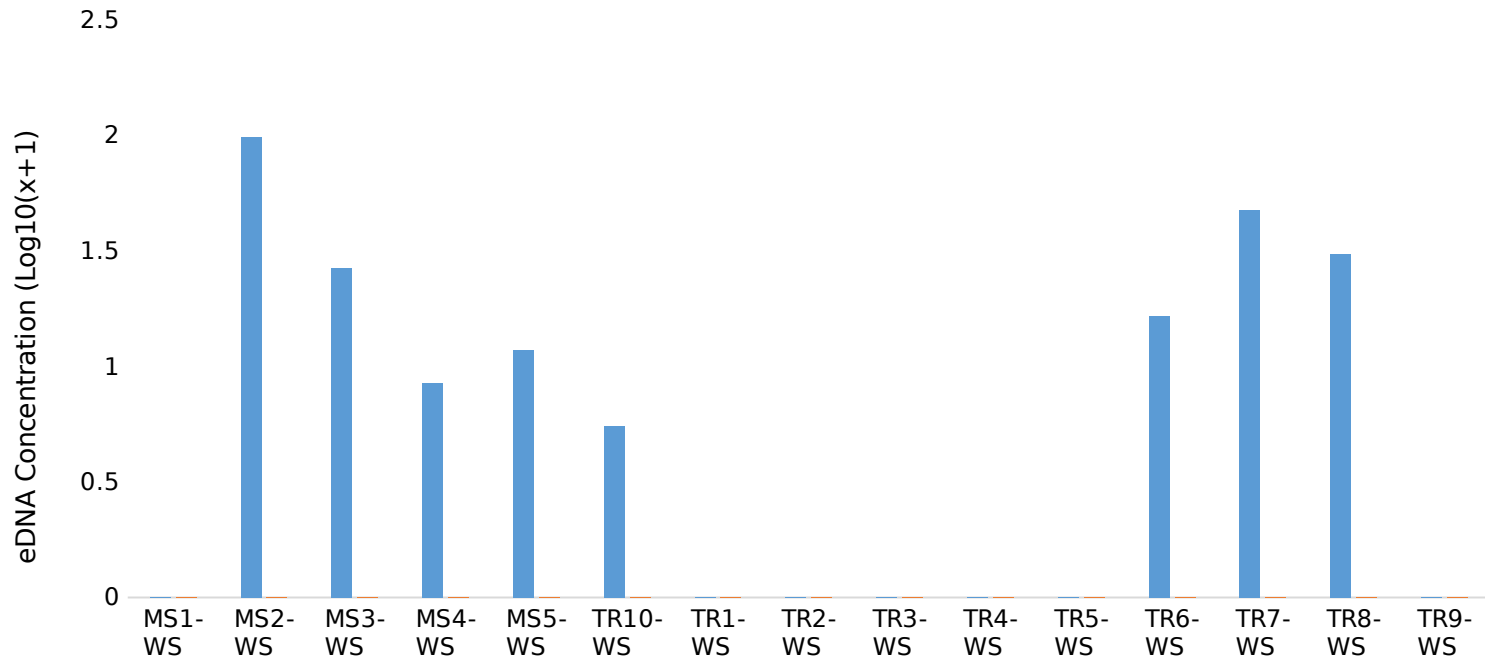
Problematic {

Coho only	9/15
Coho > Steelhead	4/15
Steelhead > Coho	0/15
Equal	0/15
No detection	2/15



1) eDNA use for species detection of redds in Hoh River tributaries?

## Water column samples: only coho eDNA detected



Coho Salmon Redd Adjacent Water Column

■ Coho Log Transformed ■ Steelhead Log Transformed

1) eDNA use for species detection of redds in Hoh River tributaries?

## eDNA concentration higher in redds than water column

Group	Species (eDNA analysis)	<i>P</i> - Value	Description
Coho Salmon redds	Coho Salmon	<b>0.0017</b>	Coho in redd > Coho in water column

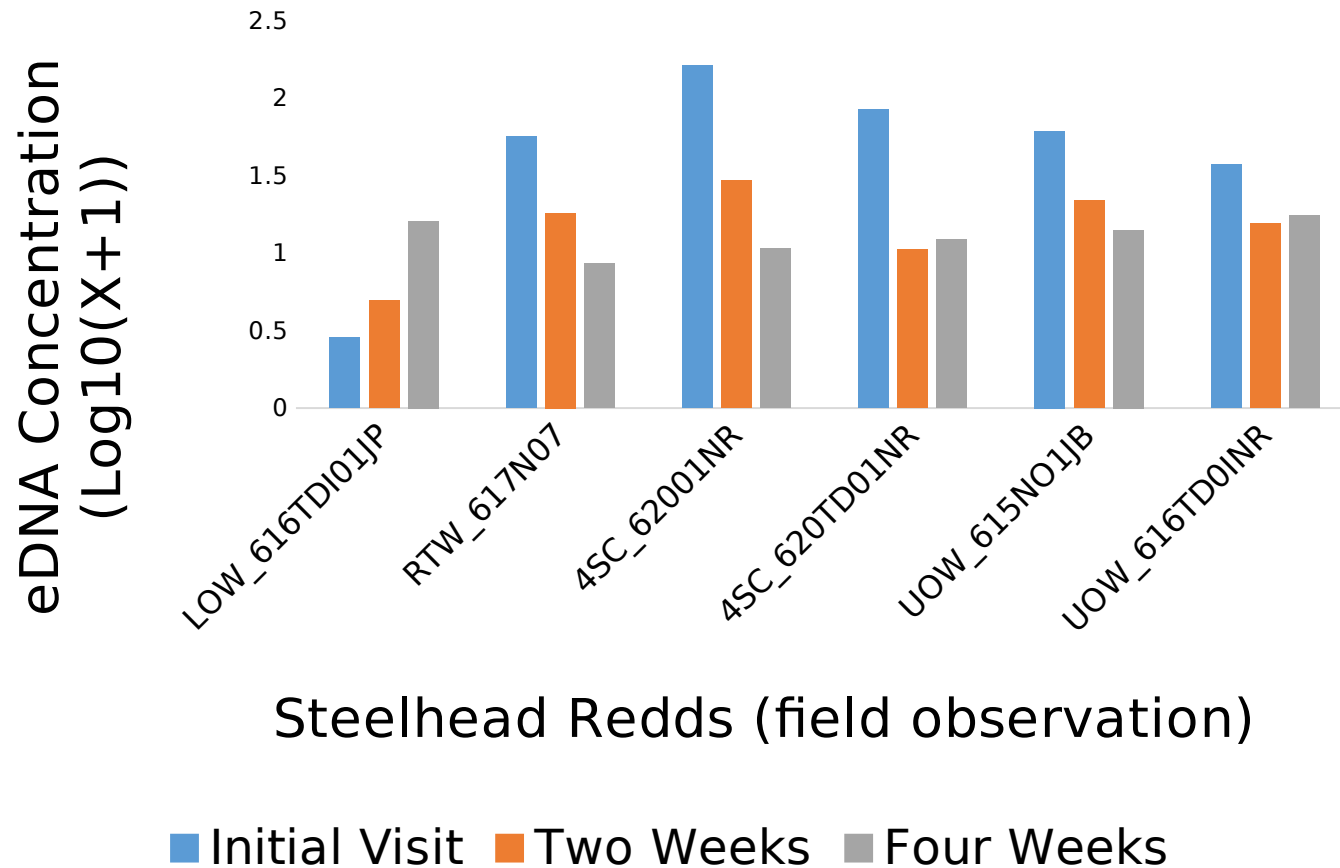
1) eDNA use for species detection of redds in Hoh River tributaries?

## eDNA concentration higher in respective species redds

Group	Species (eDNA analysis)	P- Value	Description
Steelhead redds	Coho Salmon vs. Steelhead	<b>0.0165</b>	[Steelhead] > [Coho]
Coho Salmon redds	Coho Salmon vs. Steelhead	<b>0.0017</b>	[Coho] > [Steelhead]



2) *Is there a change in eDNA concentration over time?*  
Steelhead redds: eDNA concentration detectable  
after four weeks



# Conclusions

- *1) eDNA use for species detection of redds in Hoh River tributaries?*
  - 88% of STHD and 100% of Coho redds were correctly assigned
  - Mean [eDNA] highest of field ID species
  - More mixed DNA in STHD redds than Coho
    - Coho carcasses in system when STHD are spawning?
    - Coho juveniles?
- *2) Is there a change in eDNA concentration over time?*
  - Slight decrease over time
  - Wide window of detection (>28 days)

# Future work

- Conduct steelhead sampling with adjacent water column filtered on site
  - Gravel samples
- Identify systems where this methodology will be most useful to inform uncertainties in population monitoring
  - e.g., late coho runs overlap with early winter steelhead in southwestern Washington

Thank  
you

- Nathan Rouche- WDFW
- Joe Boucher- WDFW
- John Winkowski
- Trout Unlimited
- Mitch Kissler- Laboratory Technician



- 5 STHD redds with observation of STHD on redds
  - 3 definite STHD
  - 2 had mixed species
- 2 Coho redds with observation of Coho on red
  - 1 coho in pool next to red (mixed results)
  - 1 steelhead and coho nearby, coho pair were nearby