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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
19 November 2015

U.S. Department of the Interior
U.S. Geological Survey

Outline

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

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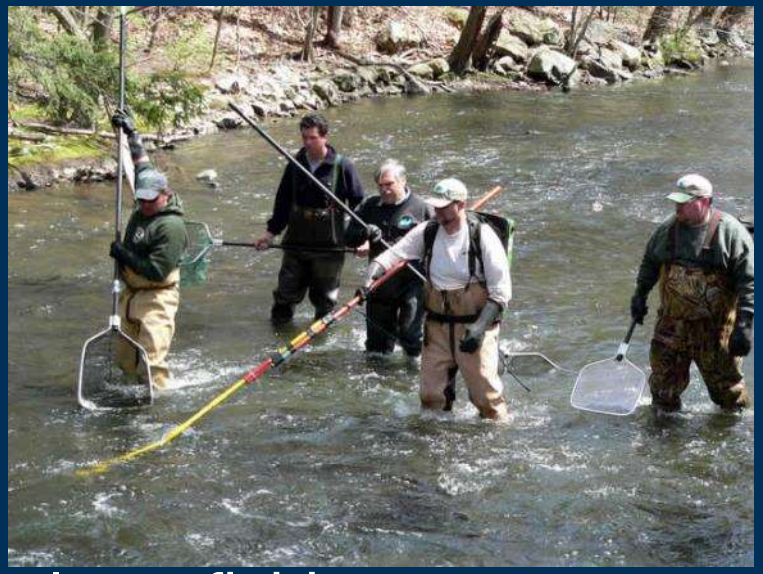
Aquatic survey methods

Photo courtesy ADF&G

Aquatic survey methods



Electrofishing

Snorkeling

USGS

Aquatic survey methods

Electrofishing

Snorkeling

eDNA

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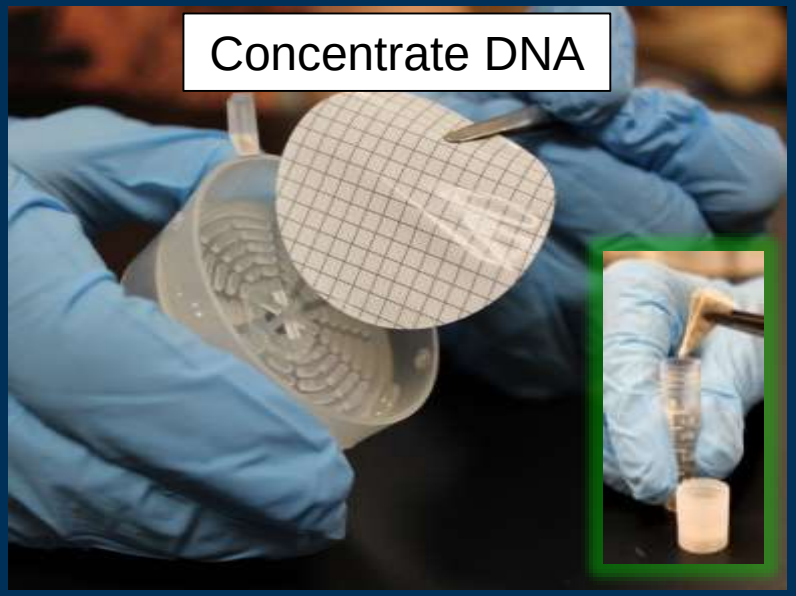
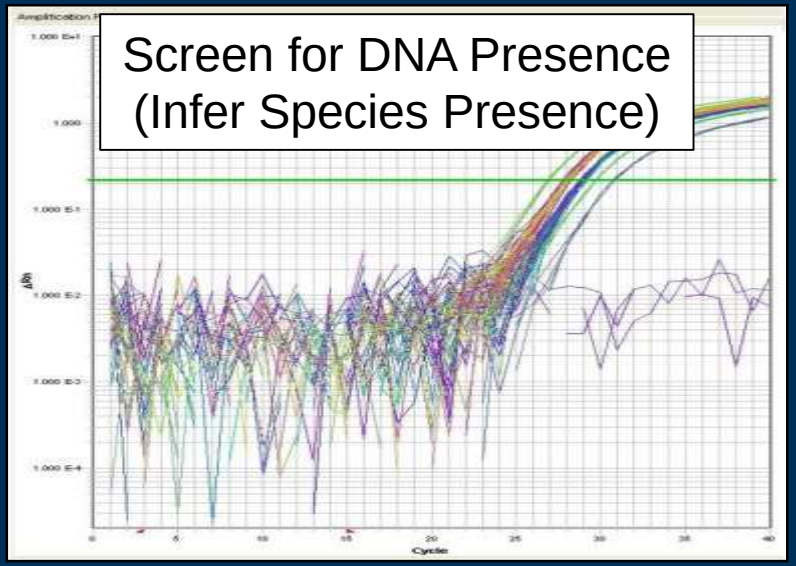
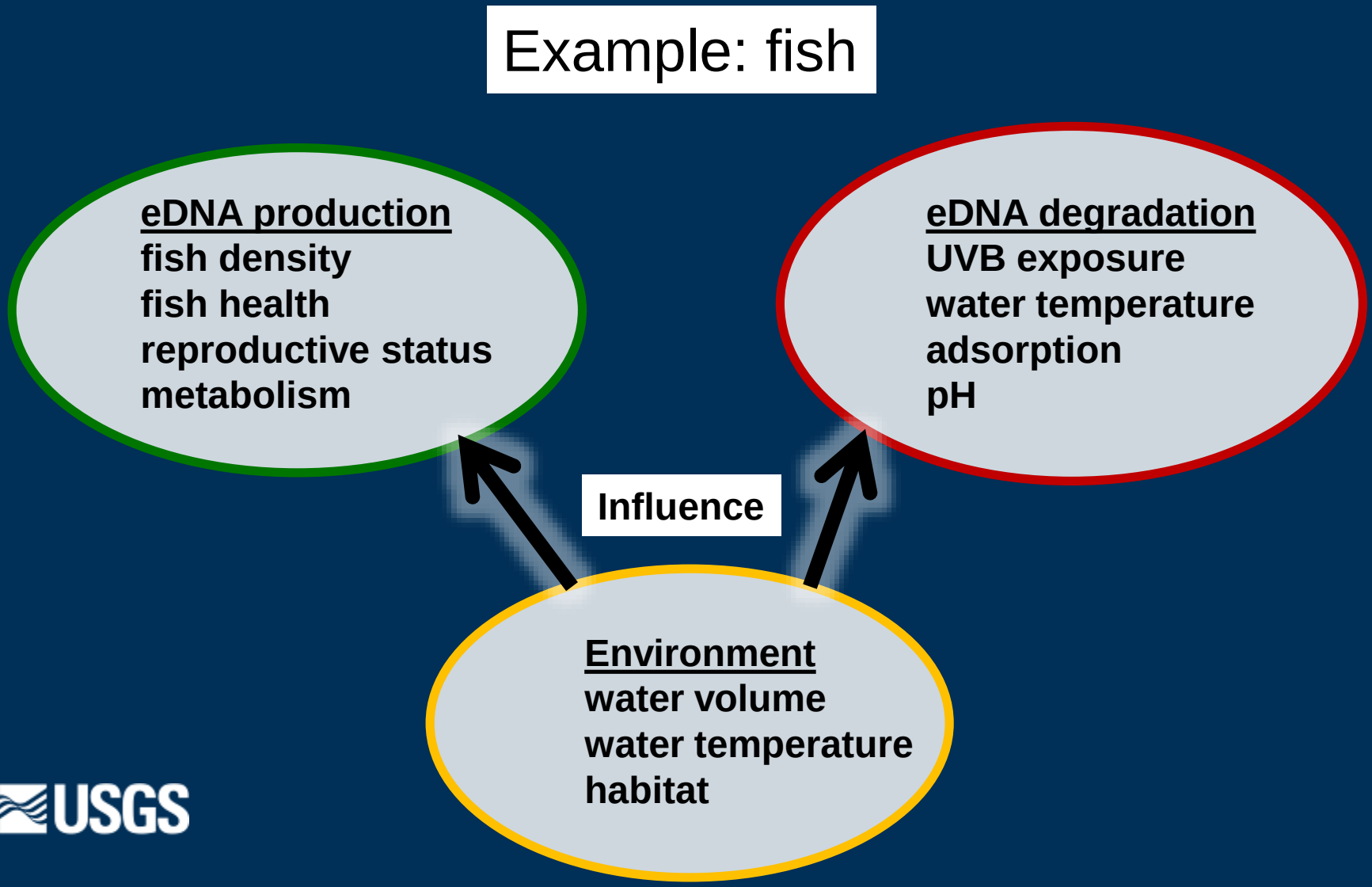
The Basic Approach





[eDNA] = production - degradation



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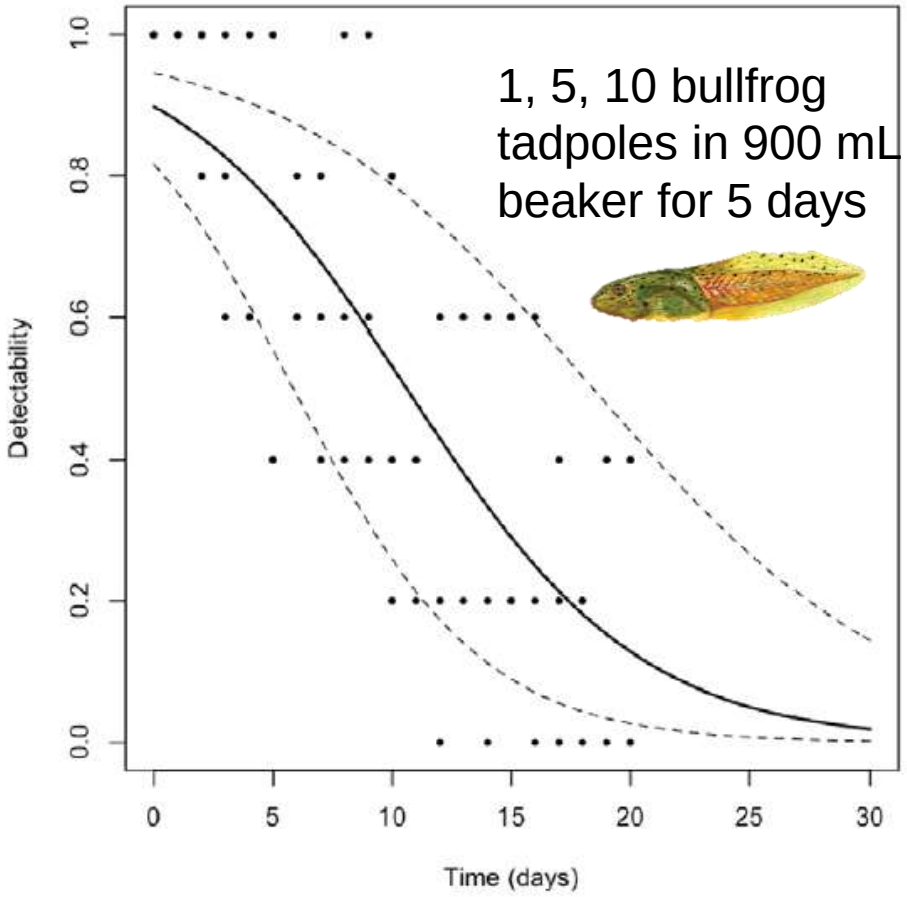
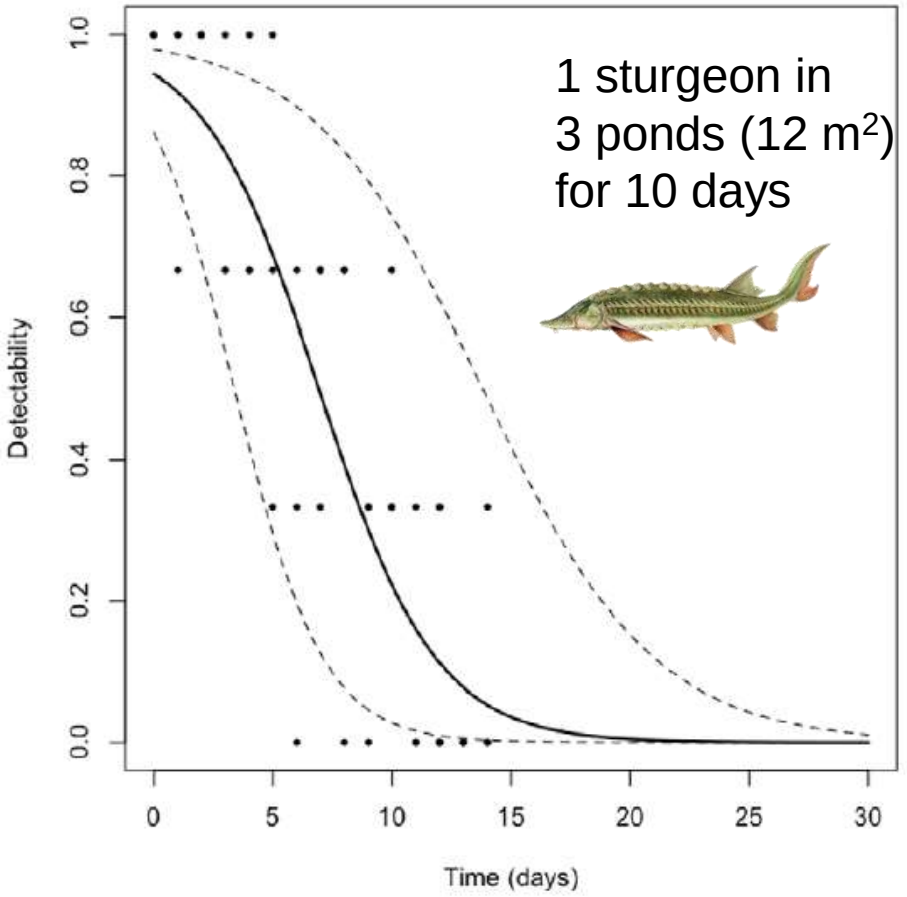
How long does DNA persist in water?





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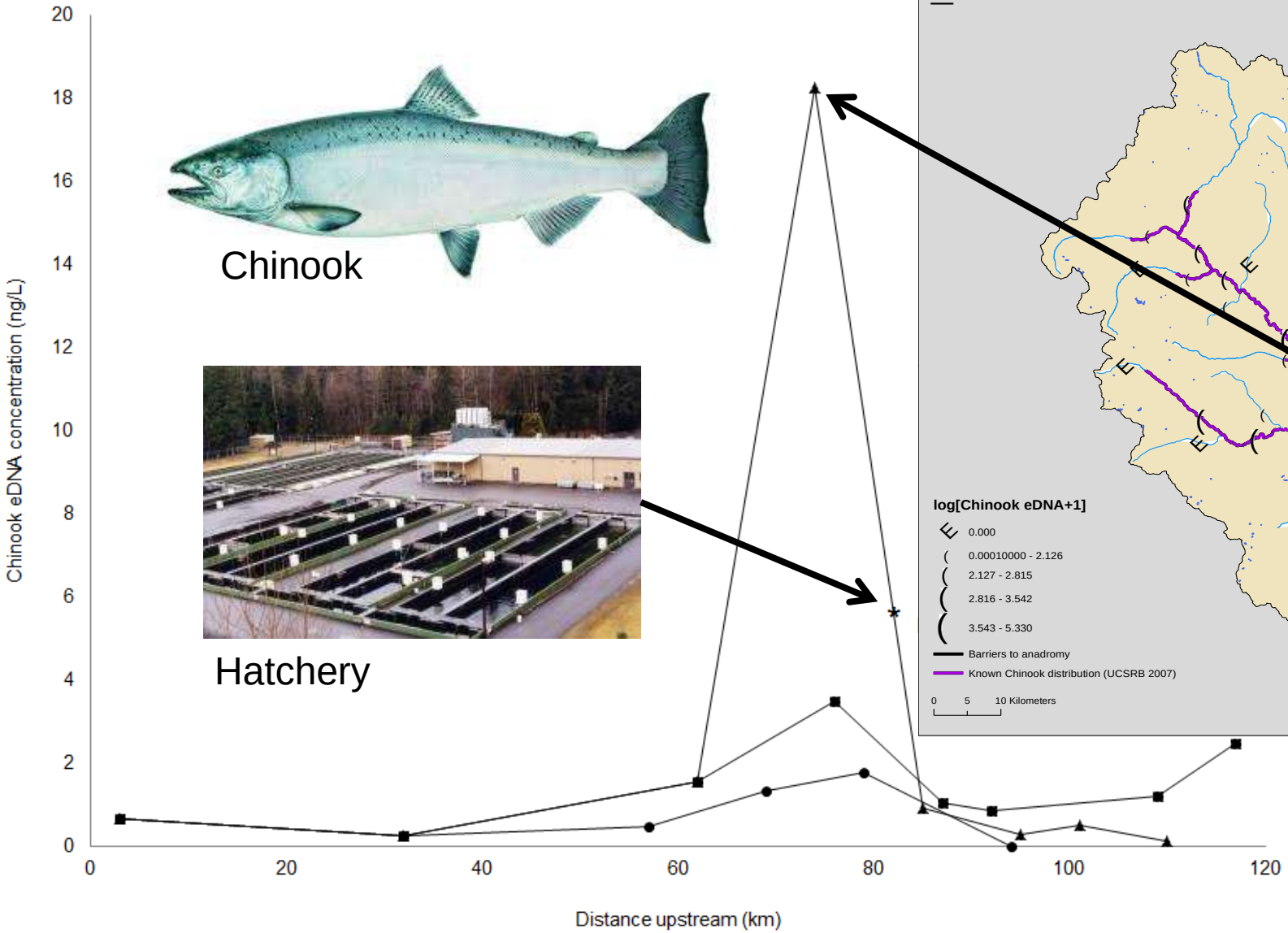
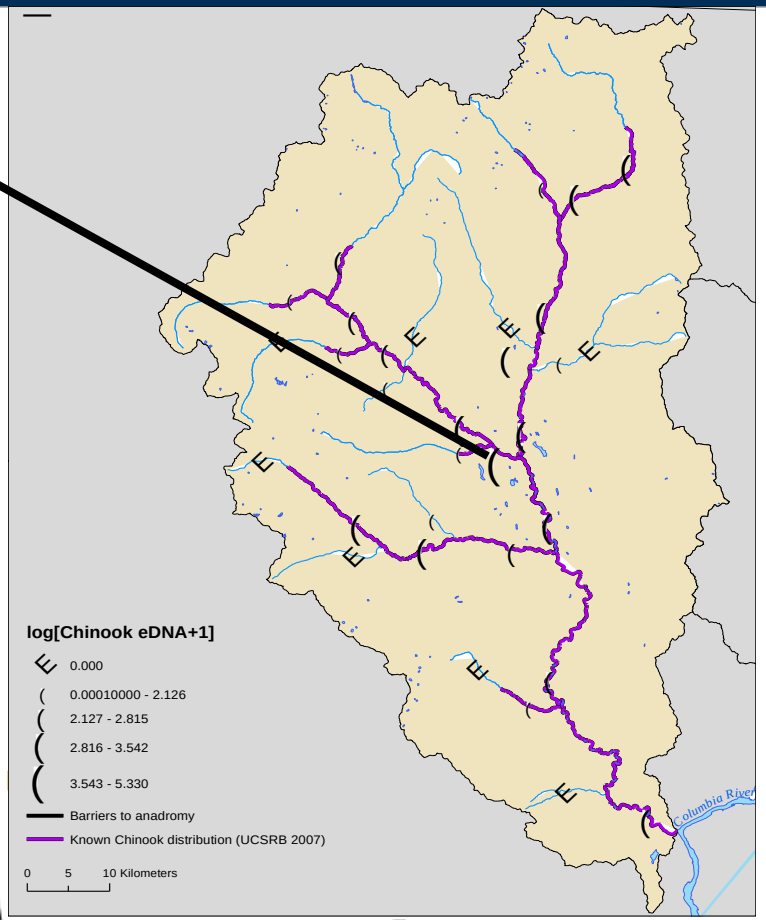
Dejean et al. (2011) PLoS One 6: e23398.

Where should samples be collected?

Chinook

Hatchery





Laramie et al. (2015) doi:10.1016/j.biocon.2014.11.025.

Integrating into Existing Monitoring Programs

Traditional survey methods

Species density		Effort required for detection
High	⟷	Low
Low	⟷	High

eDNA survey methods

Species density		Effort required for detection
High	⟷	Low
Low	⟷	Low

***Highly sensitive eDNA methods could be useful alternative to investing high effort**

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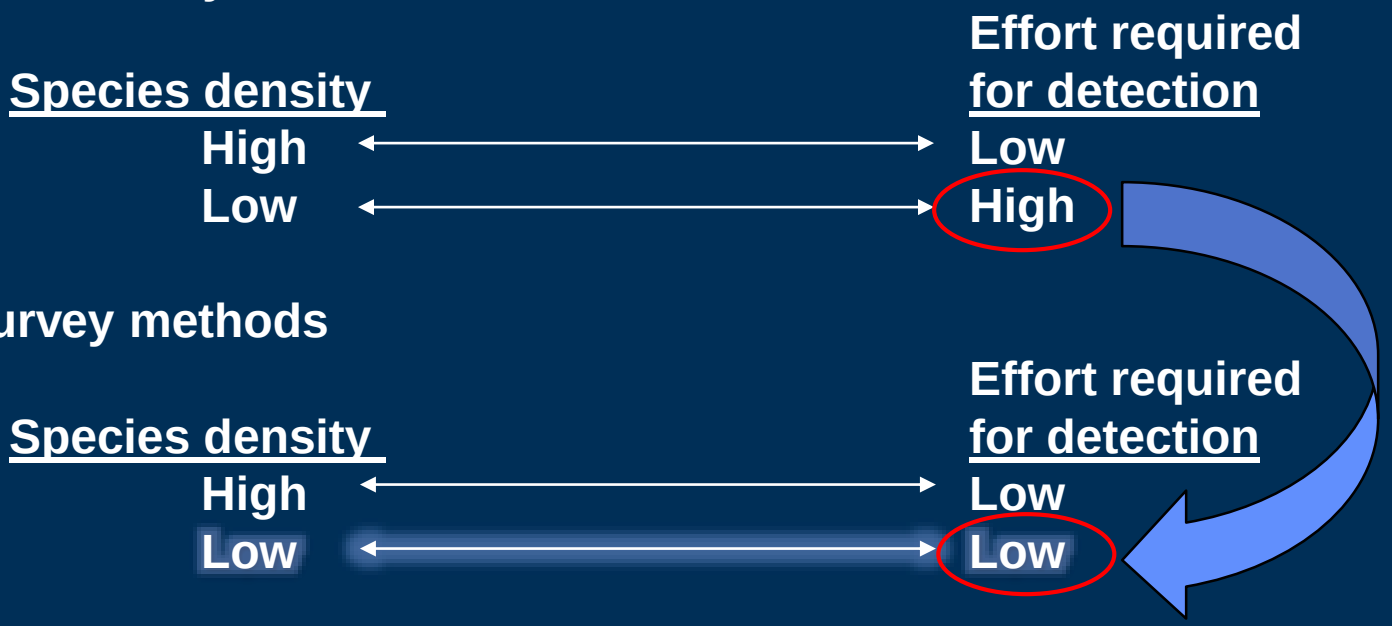
Integrating into Existing Monitoring Programs

Traditional survey methods



eDNA survey methods

***Highly sensitive eDNA methods could be useful alternative to investing high effort**

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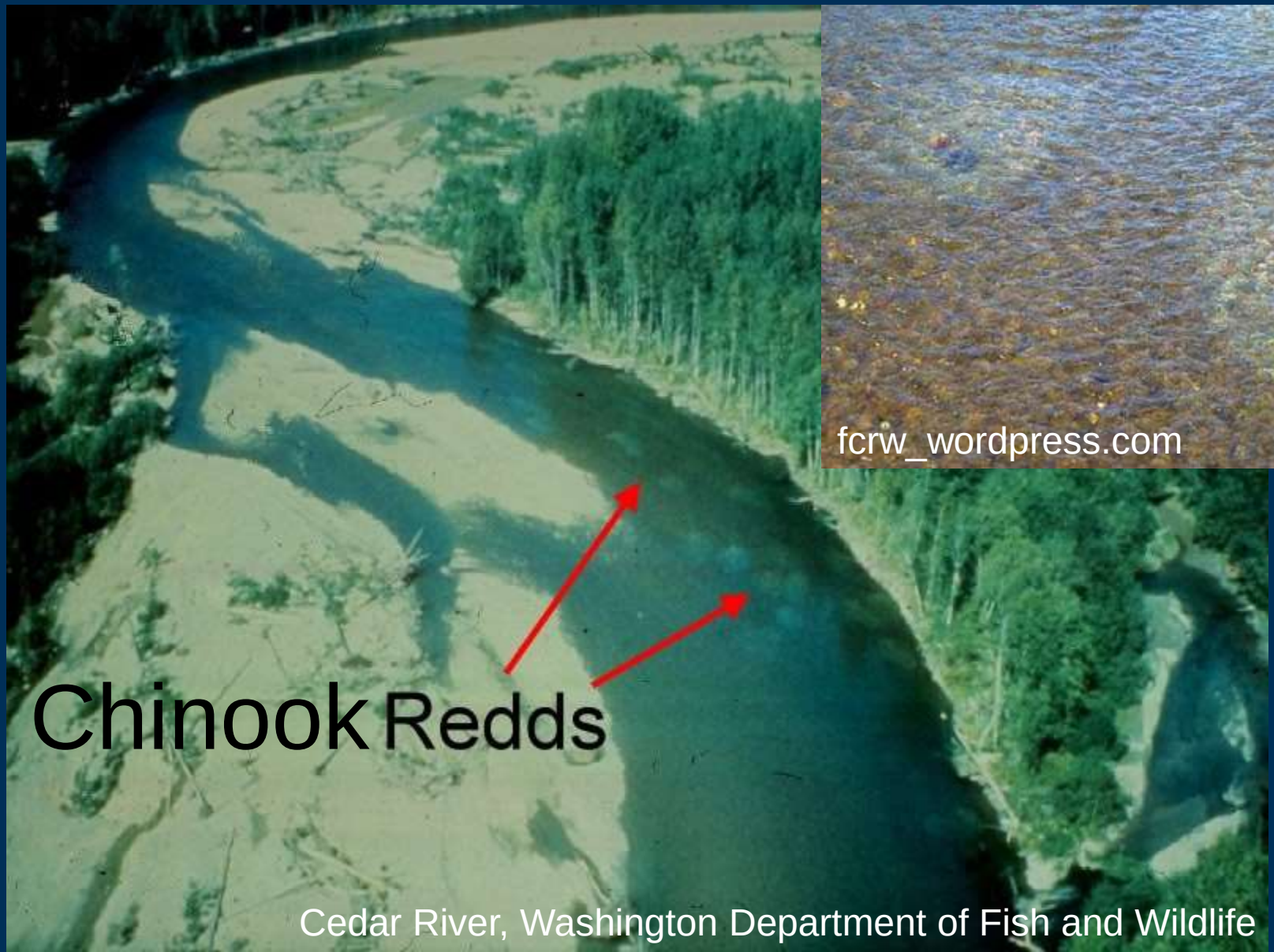
Monitoring Salmon Populations

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

Monitoring Salmon Populations

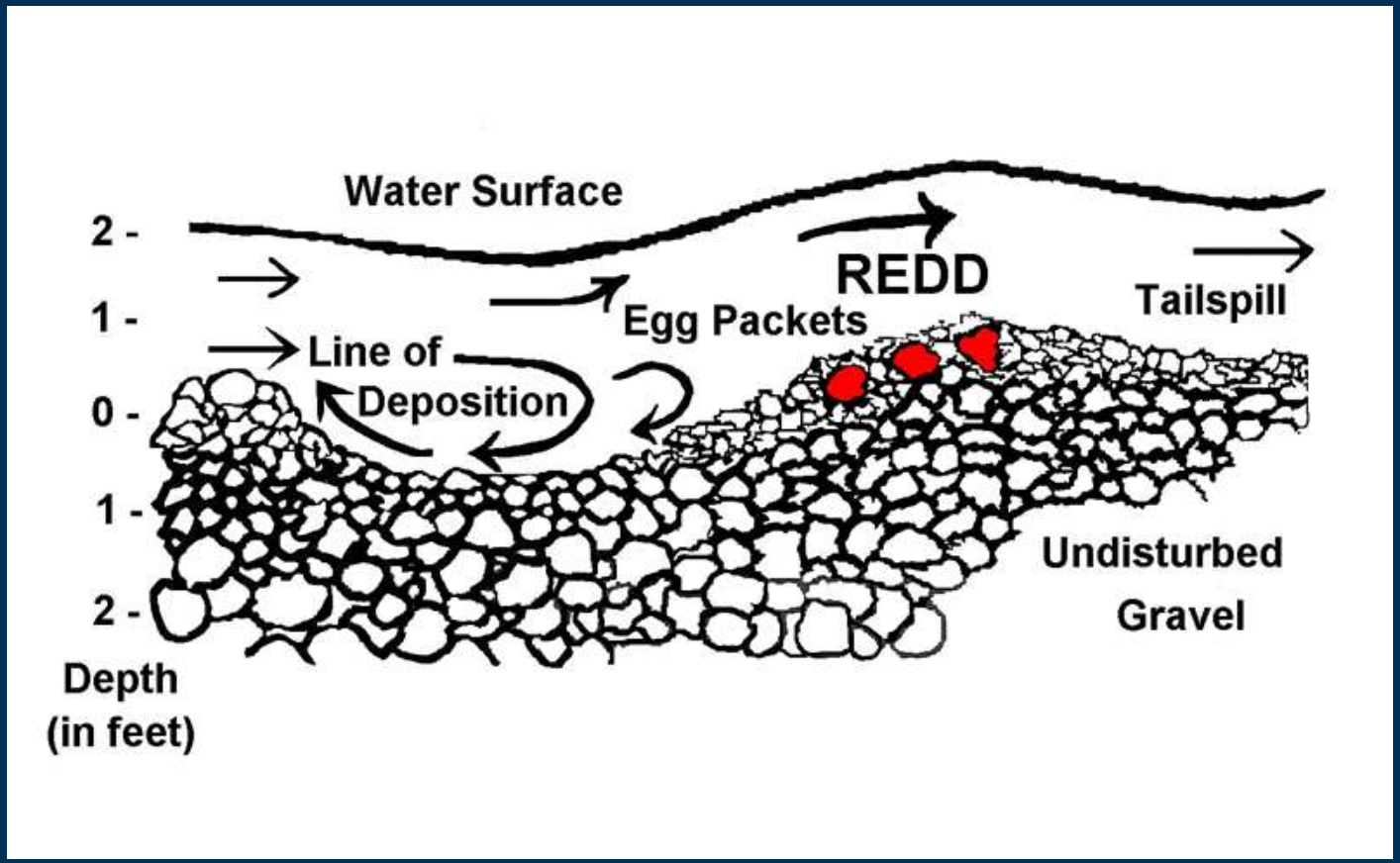


Habitat & timing used to differentiate redds where species co-occur


PORTLAND
WATER
BUREAU
FROM FOREST TO FAUCET

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?



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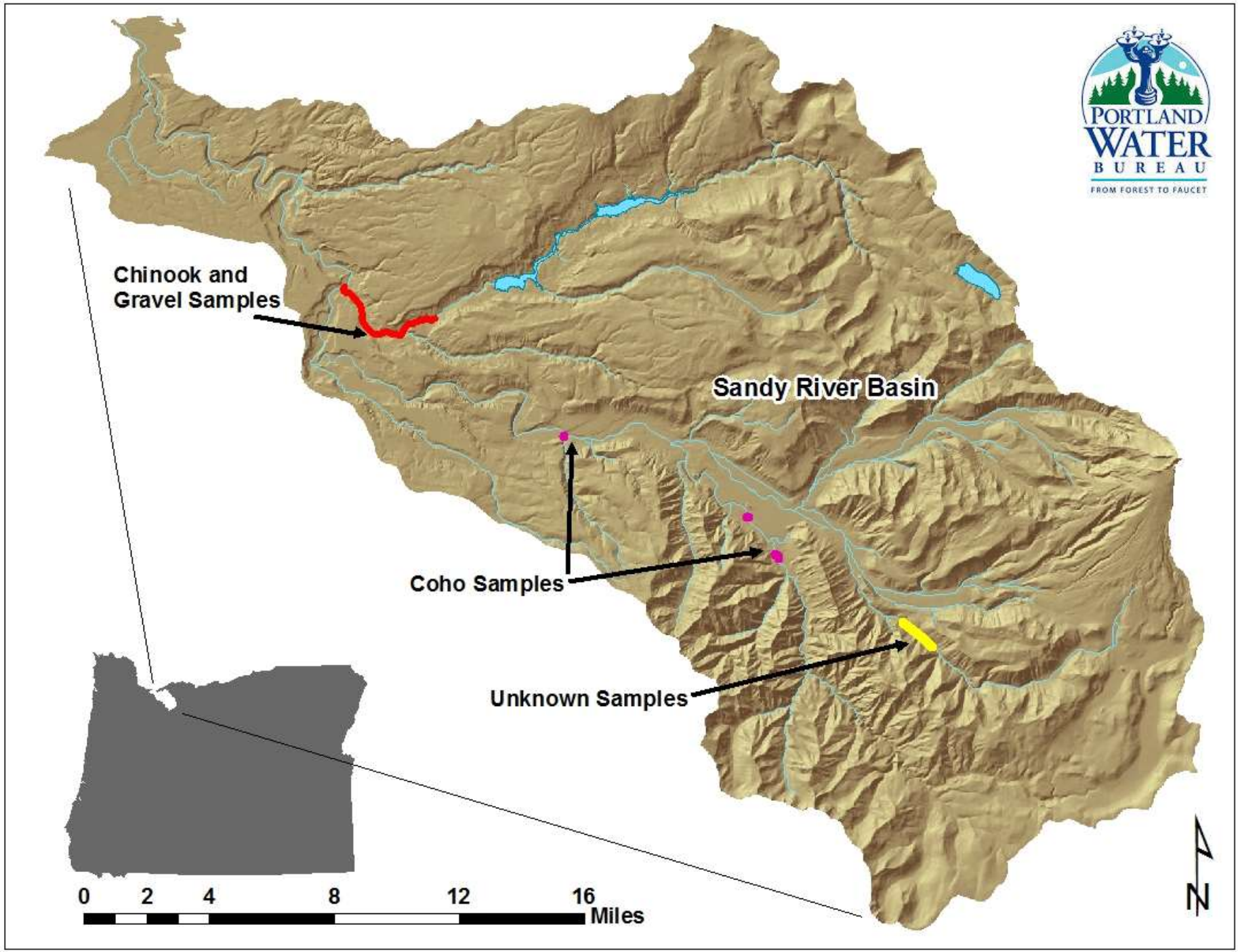
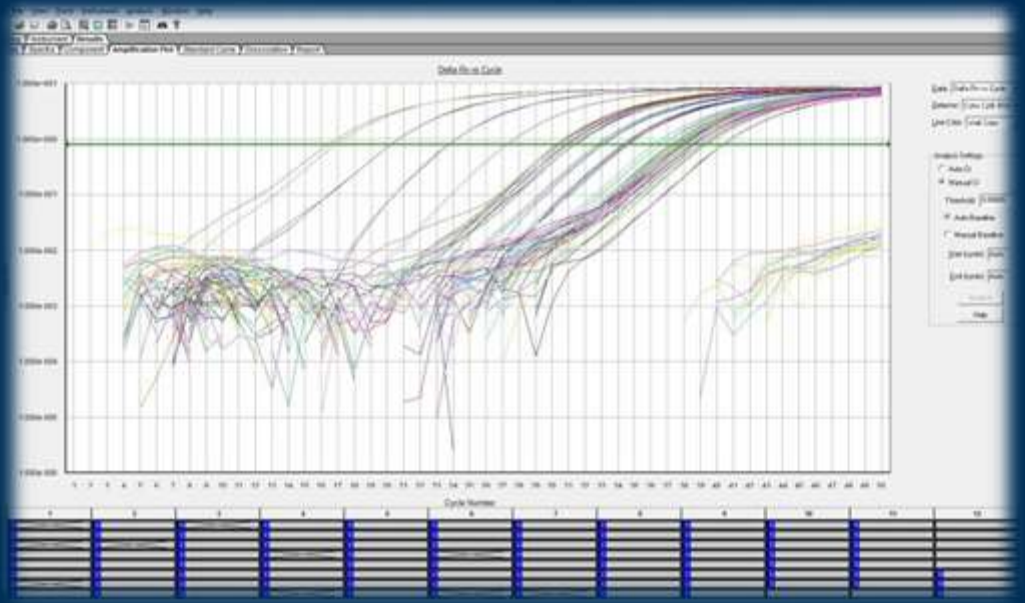

PORTLAND WATER BUREAU
FROM FOREST TO FAUCET
Chinook and Gravel Samples
Sandy River Basin
Coho Samples
Unknown Samples
0 2 4 8 12 16
Miles
N

Burke Strobel,
Portland Water Bureau

PORTLAND WATER BUREAU
FROM FOREST TO FAUCET

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



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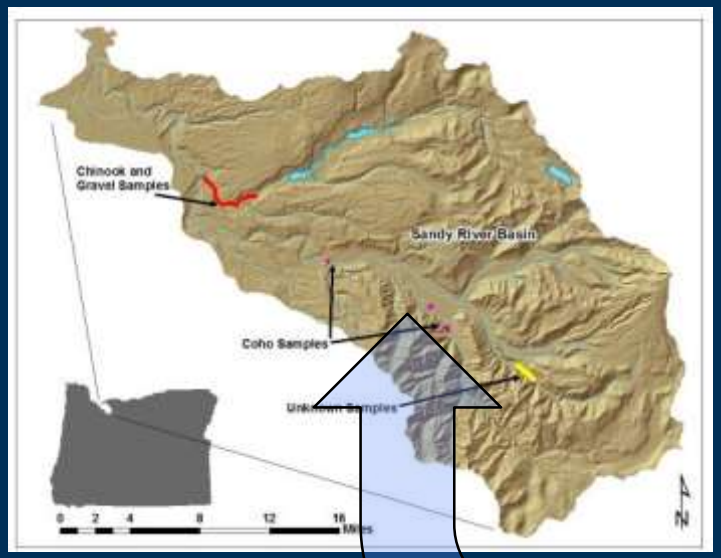
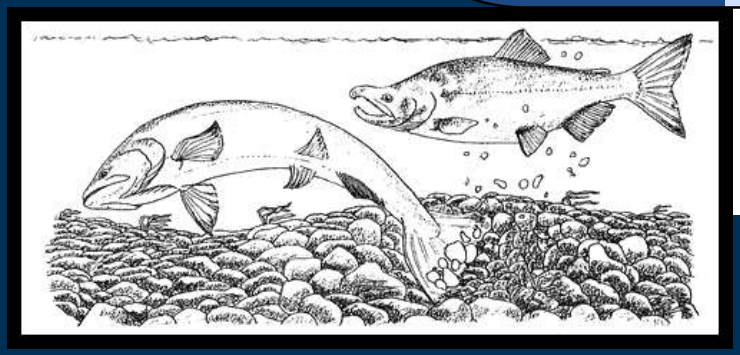
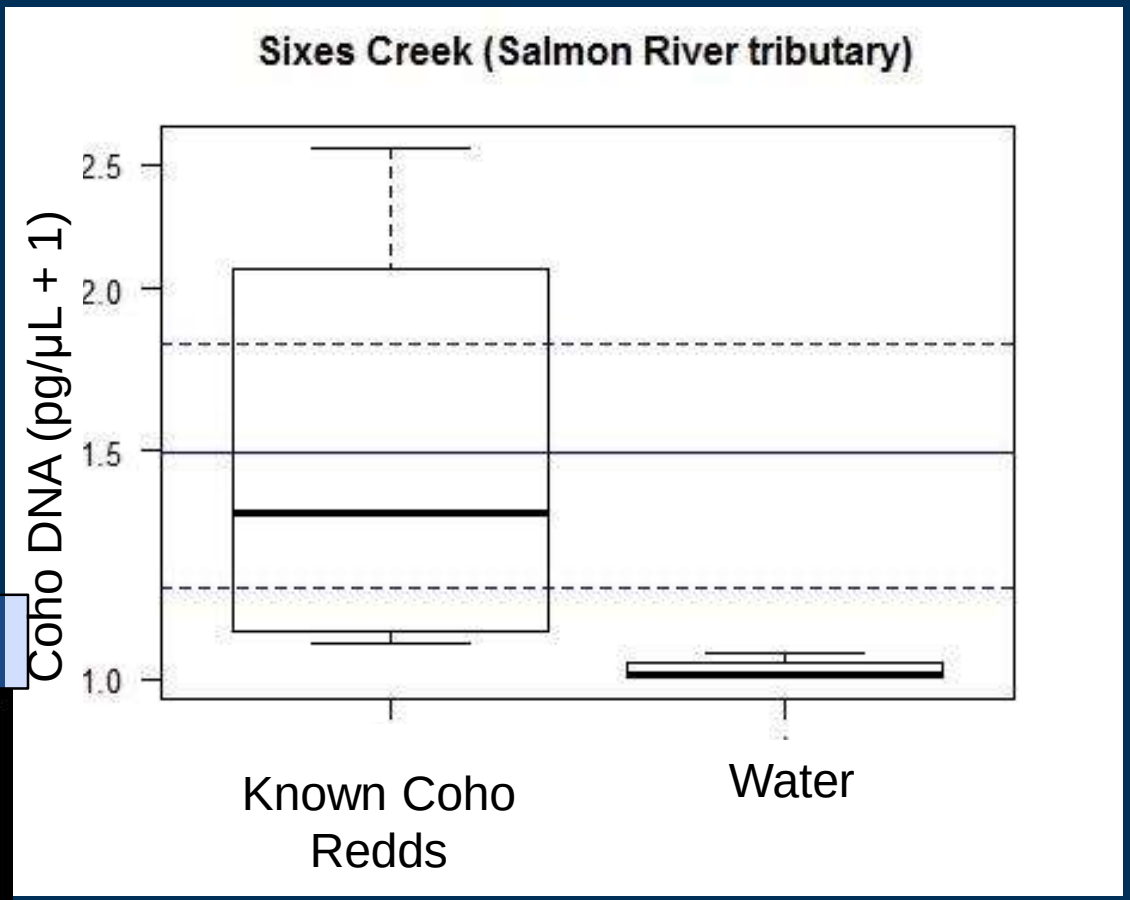
How much Coho DNA is at a Coho Redd?







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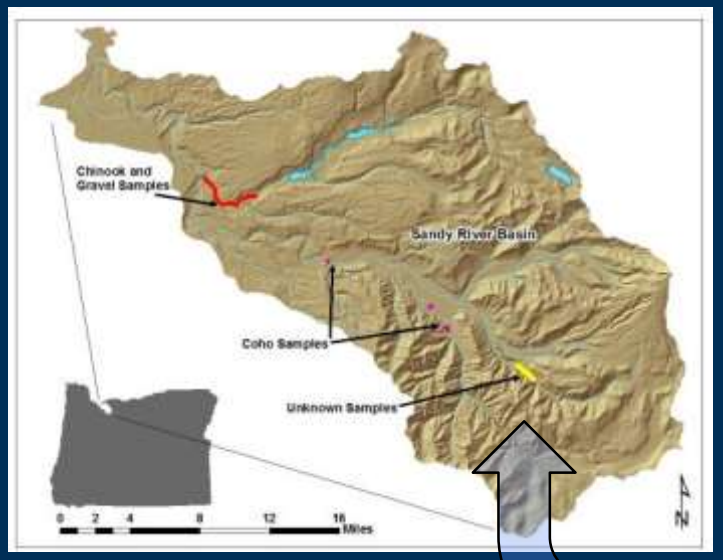
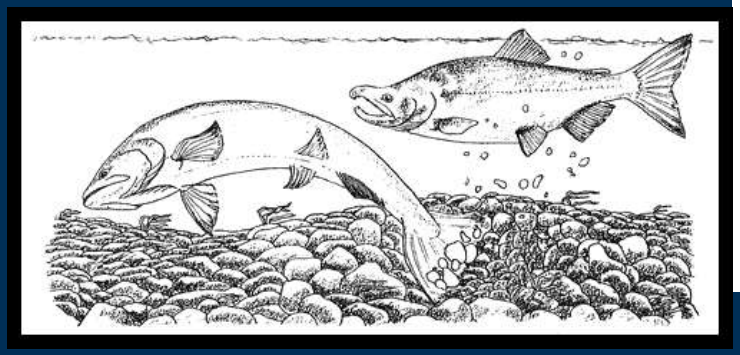
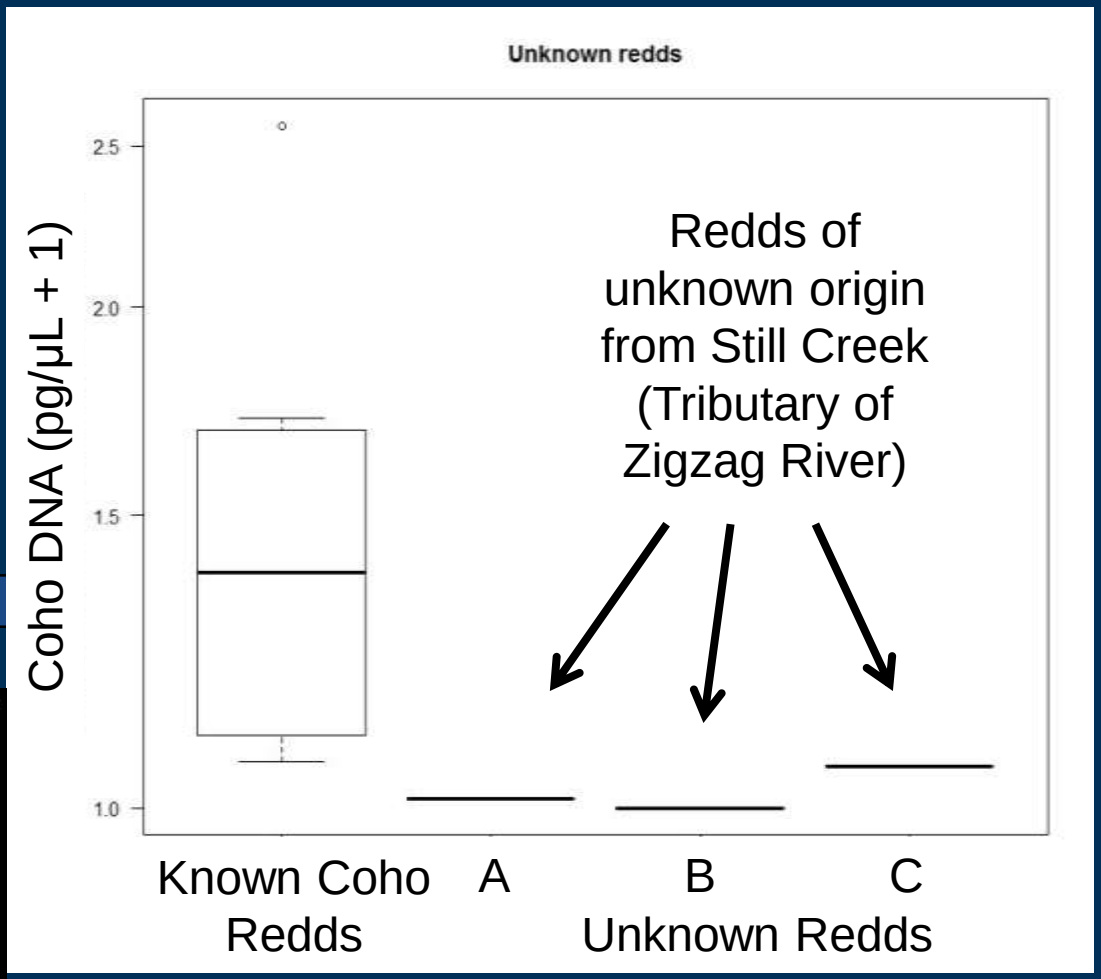
Can unknown redds be assigned?







USGS

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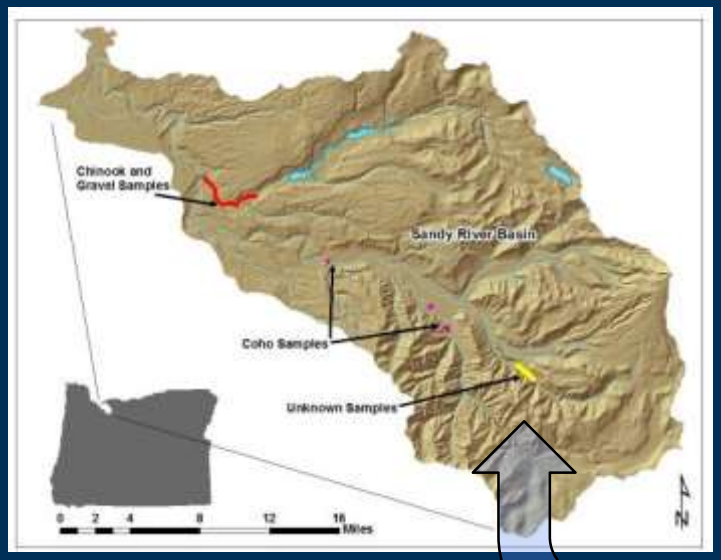

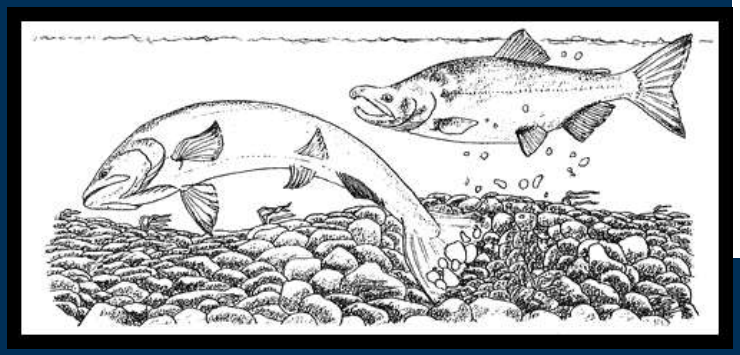

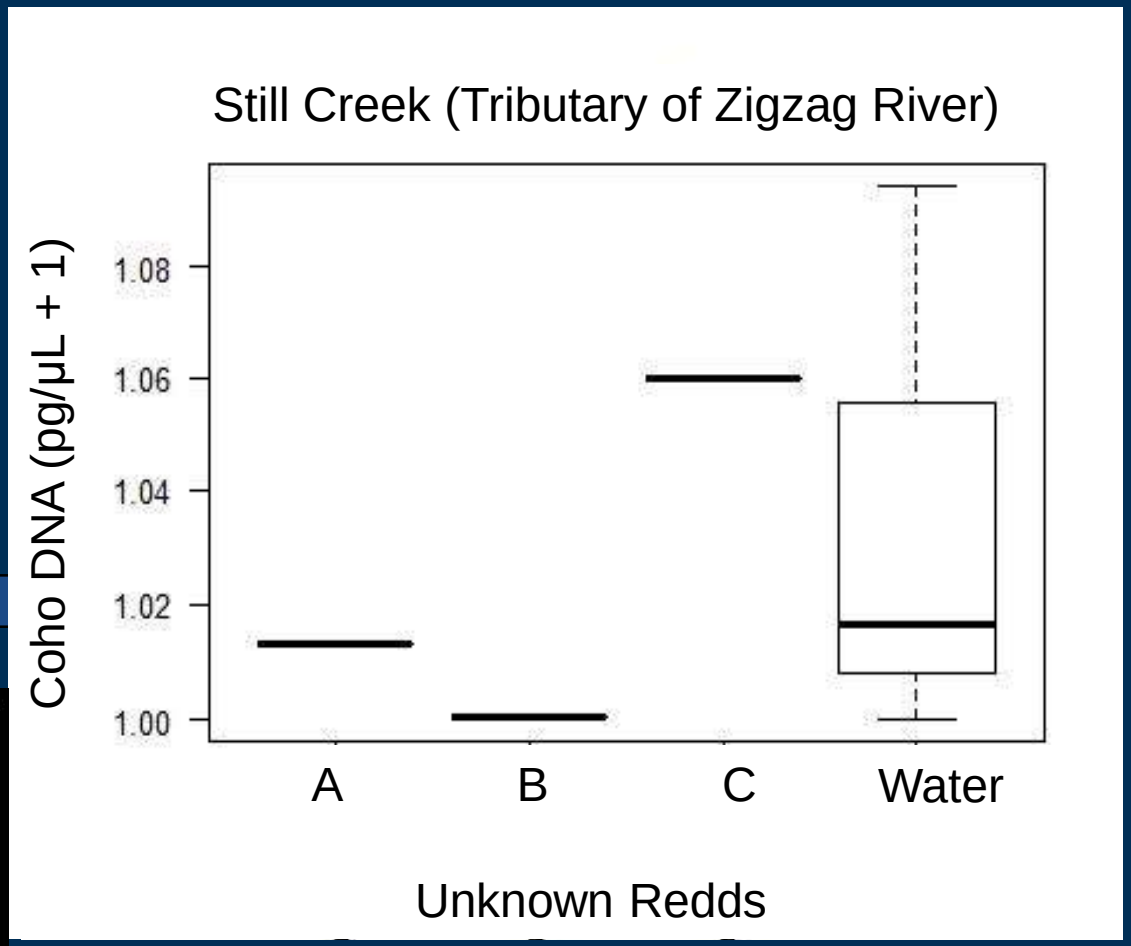

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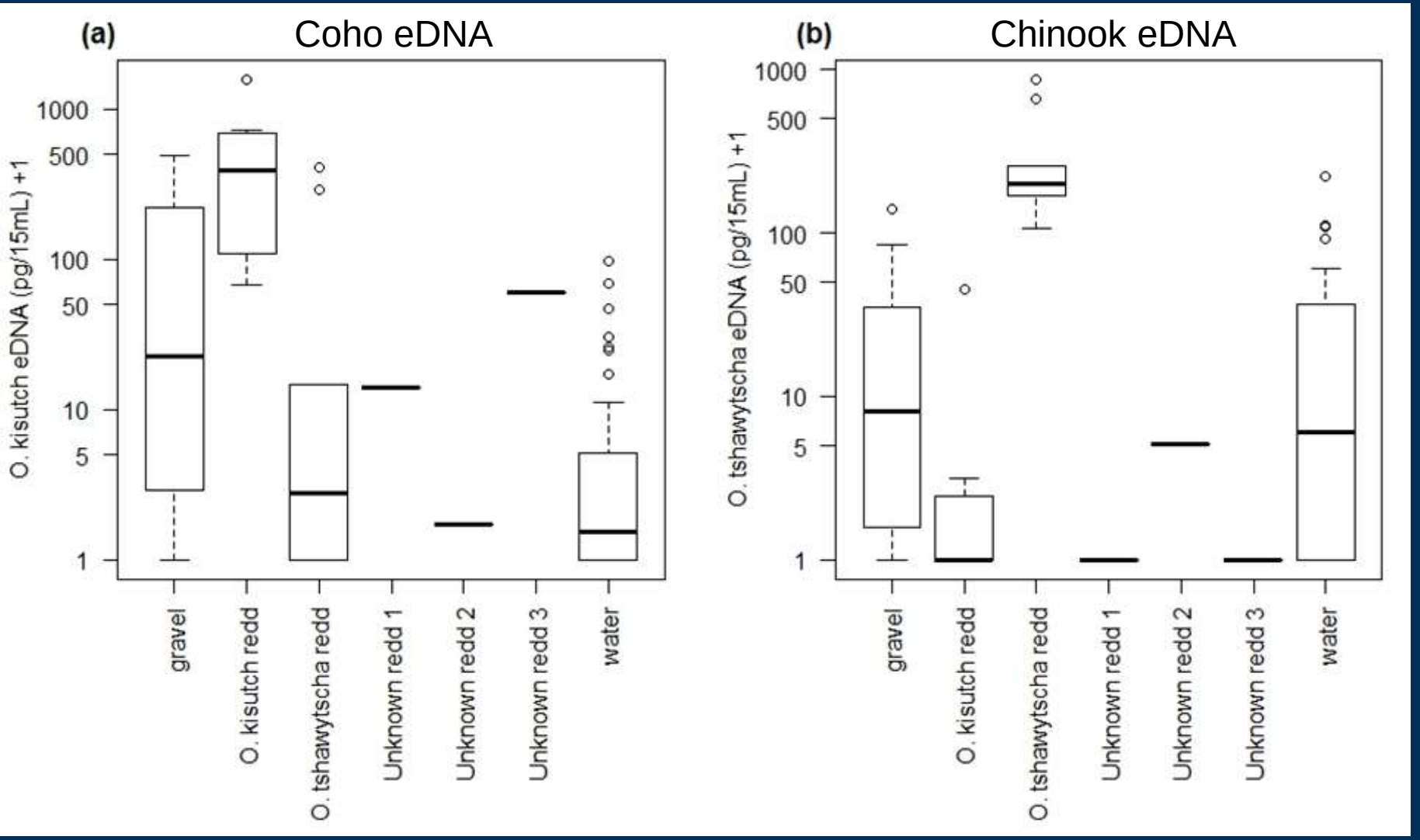
All streams combined



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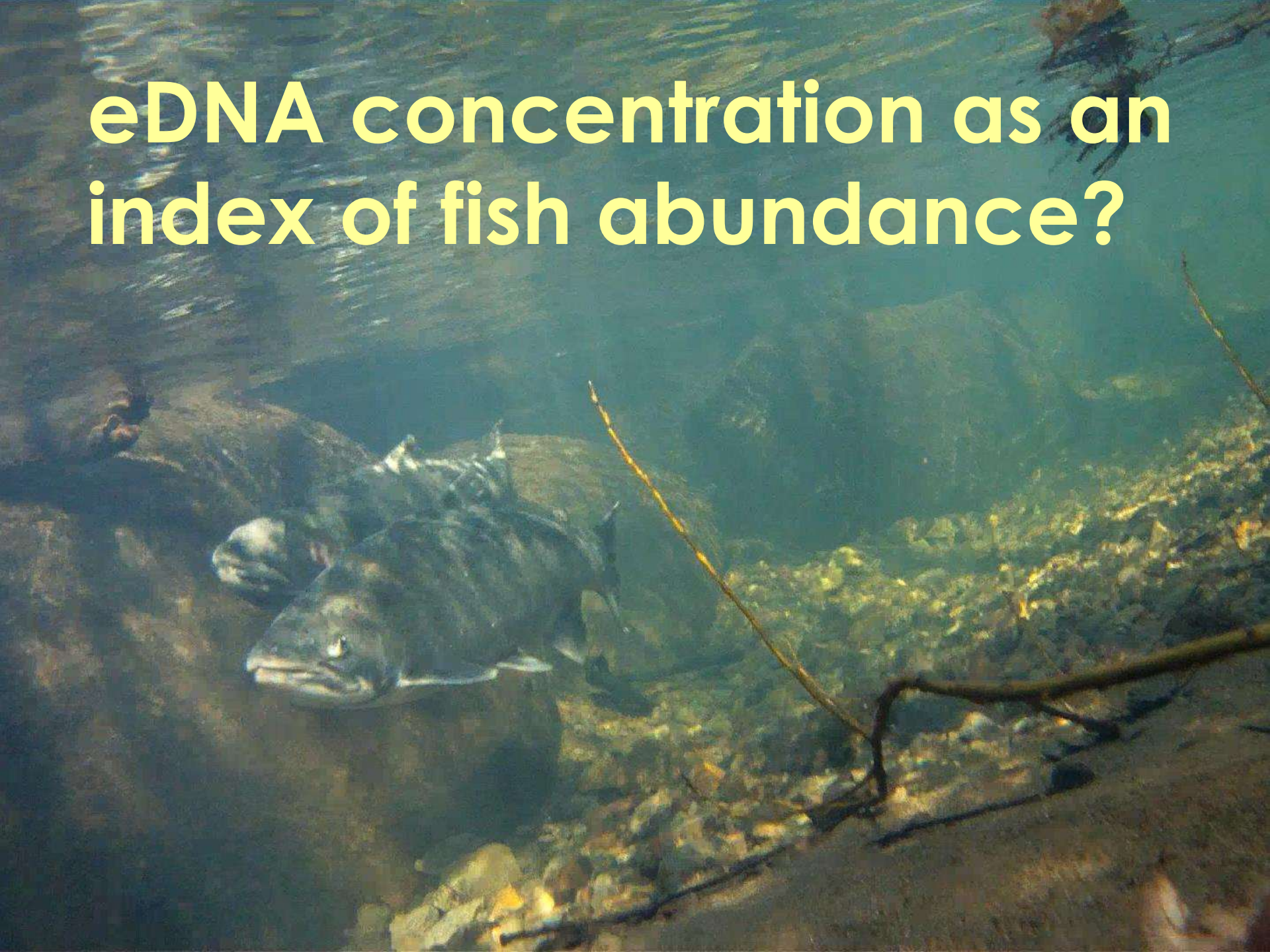
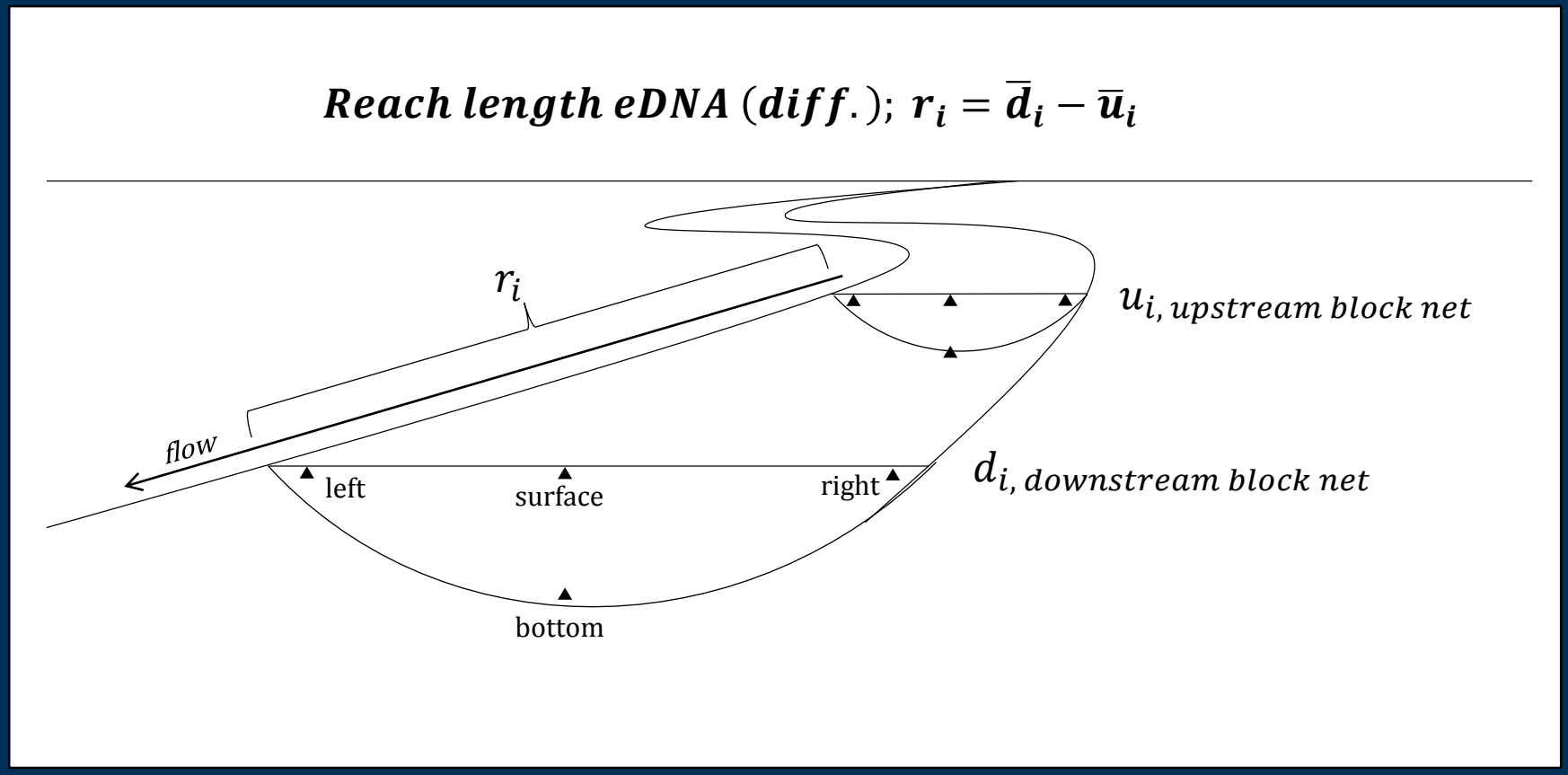
eDNA concentration as an index of fish abundance?



Omak Creek

- Mid-size perennial stream
- ~5 m wetted width
- 10 - 150cfs
- USGS Gage 12445900

1. Does [eDNA] reflect relative fish abundance?
2. Does it matter where samples are collected (cross-section)?



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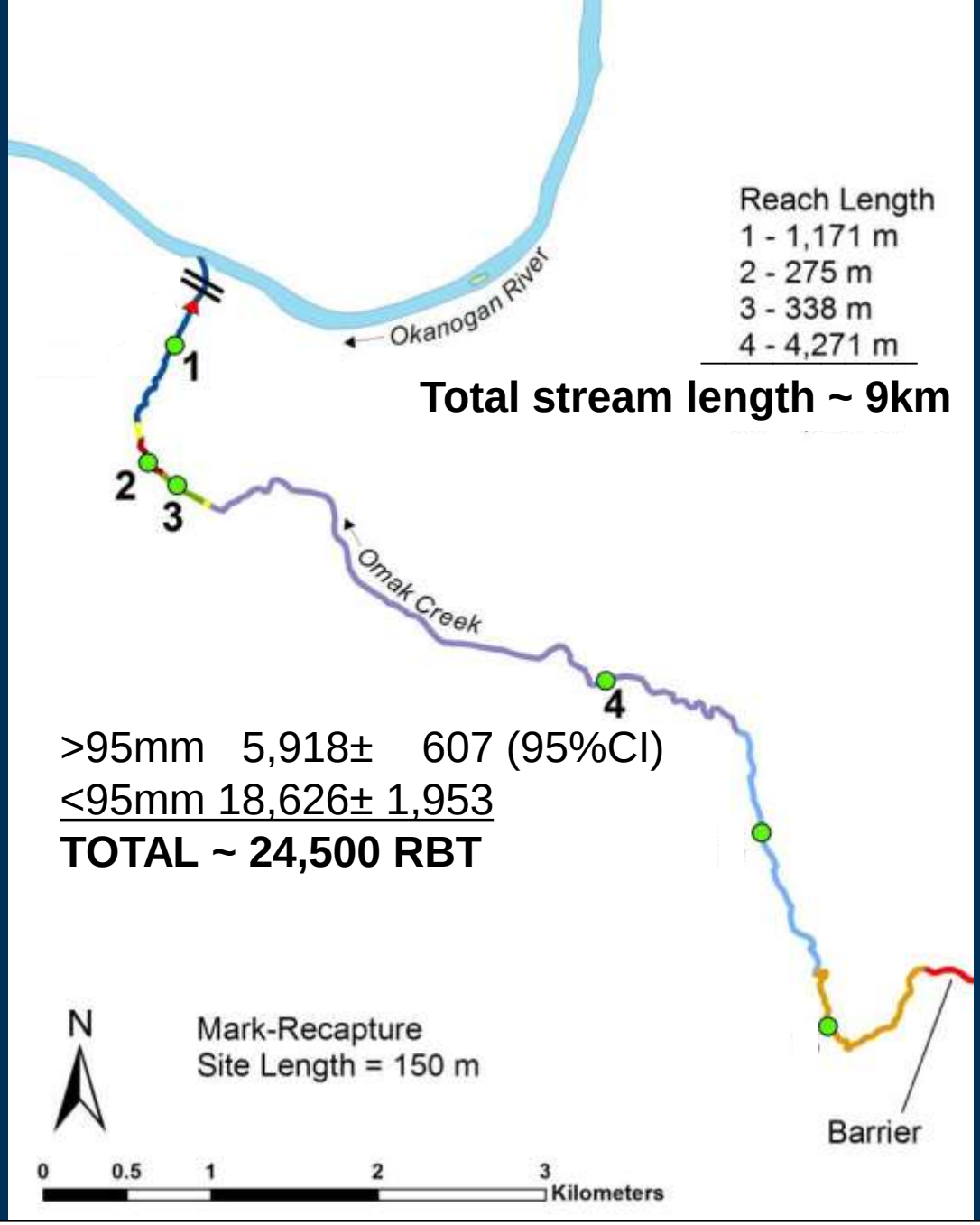
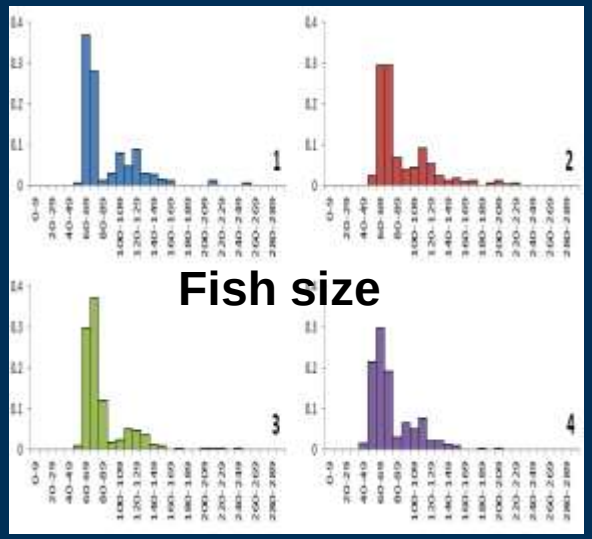
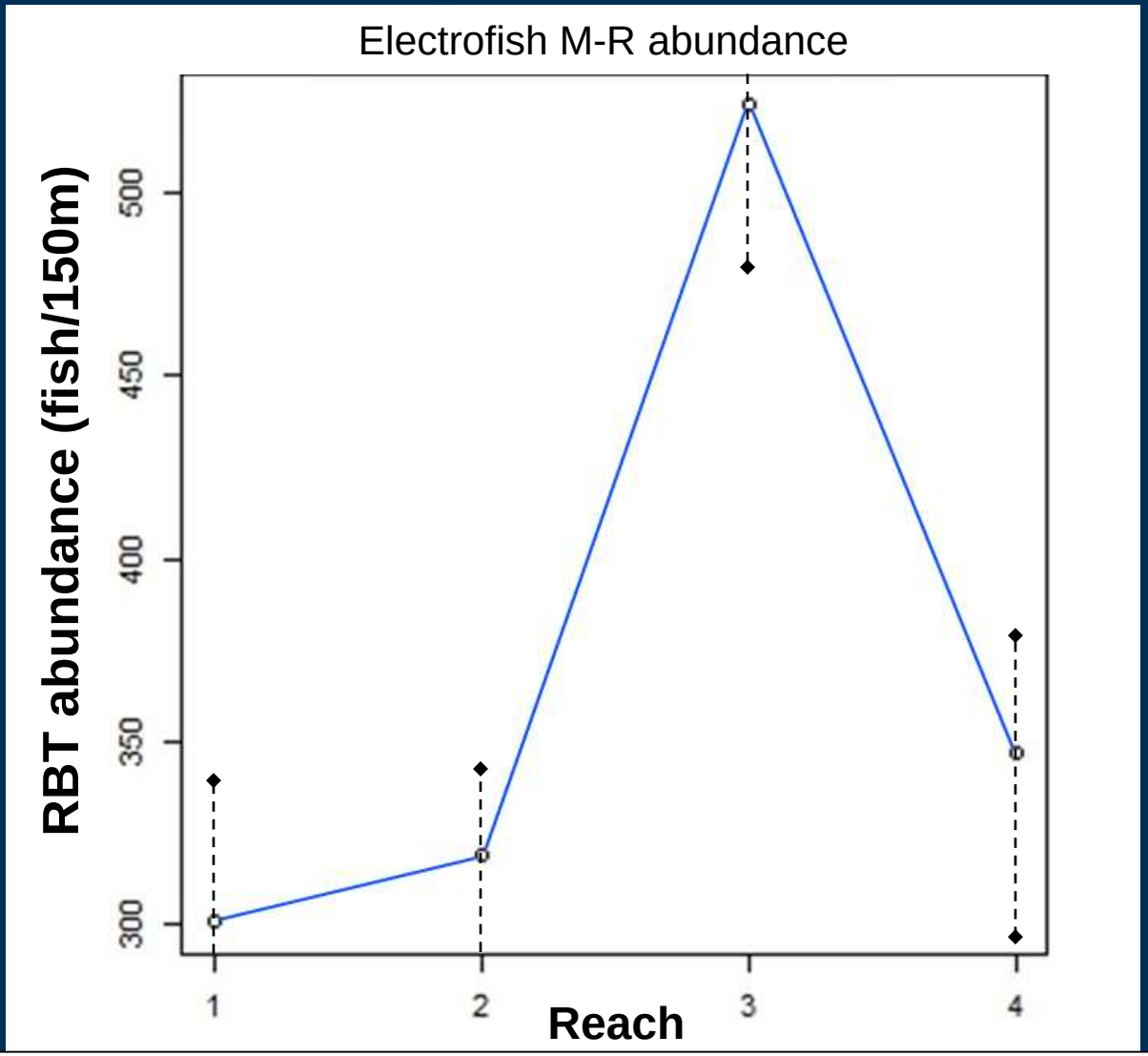


Miller, B.F., J.L. Miller, S.T. Schaller, and J.A. Arterburn. 2013. Okanogan Basin Monitoring and Evaluation Program, 2012 Annual Report. Colville Confederated Tribes Fish and Wildlife Department, Nespelem, WA. Project No. 2003-022-00.

Electrofish mark-recap RBT abundance





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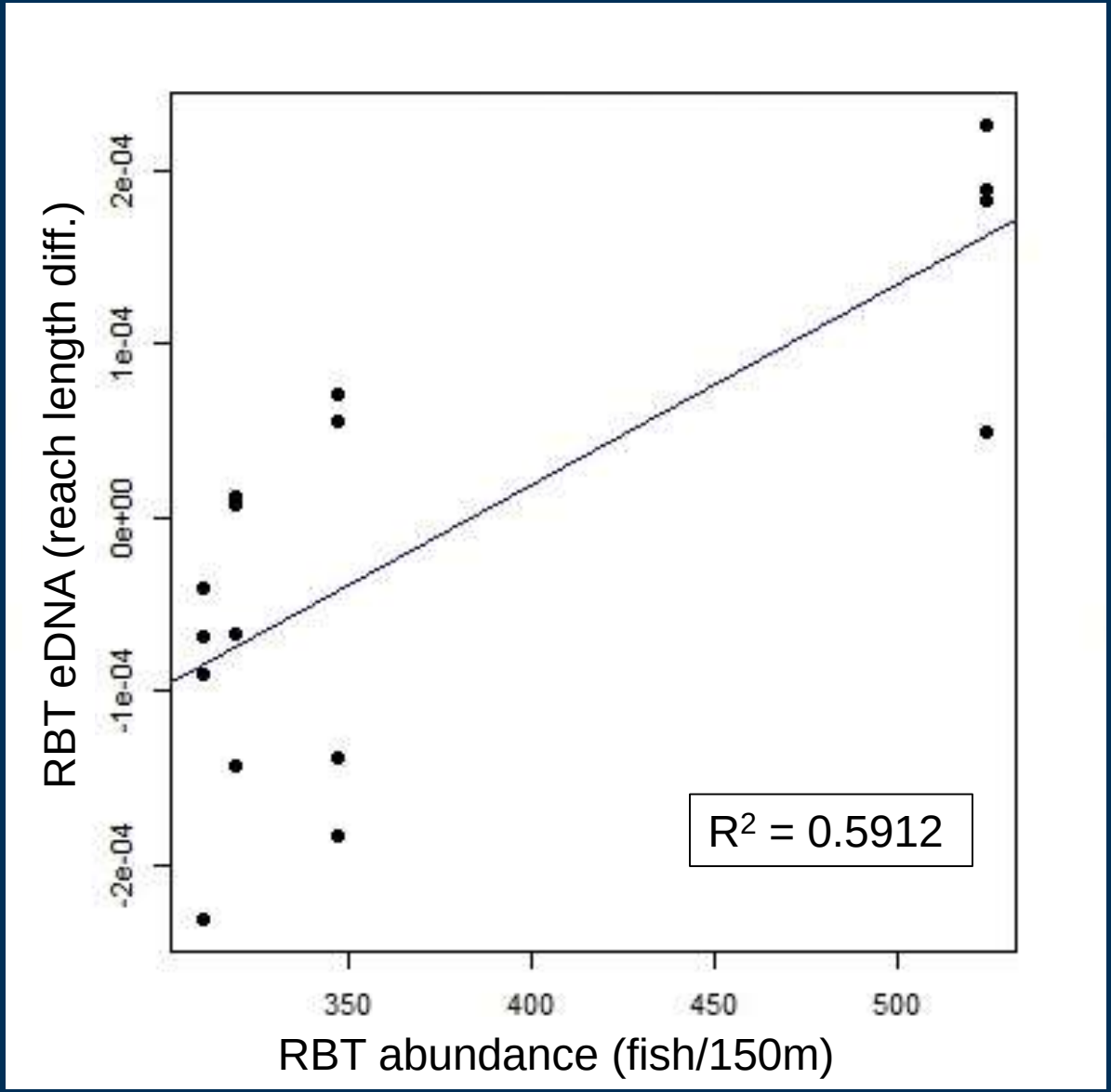
Does [eDNA] reflect fish abundance?



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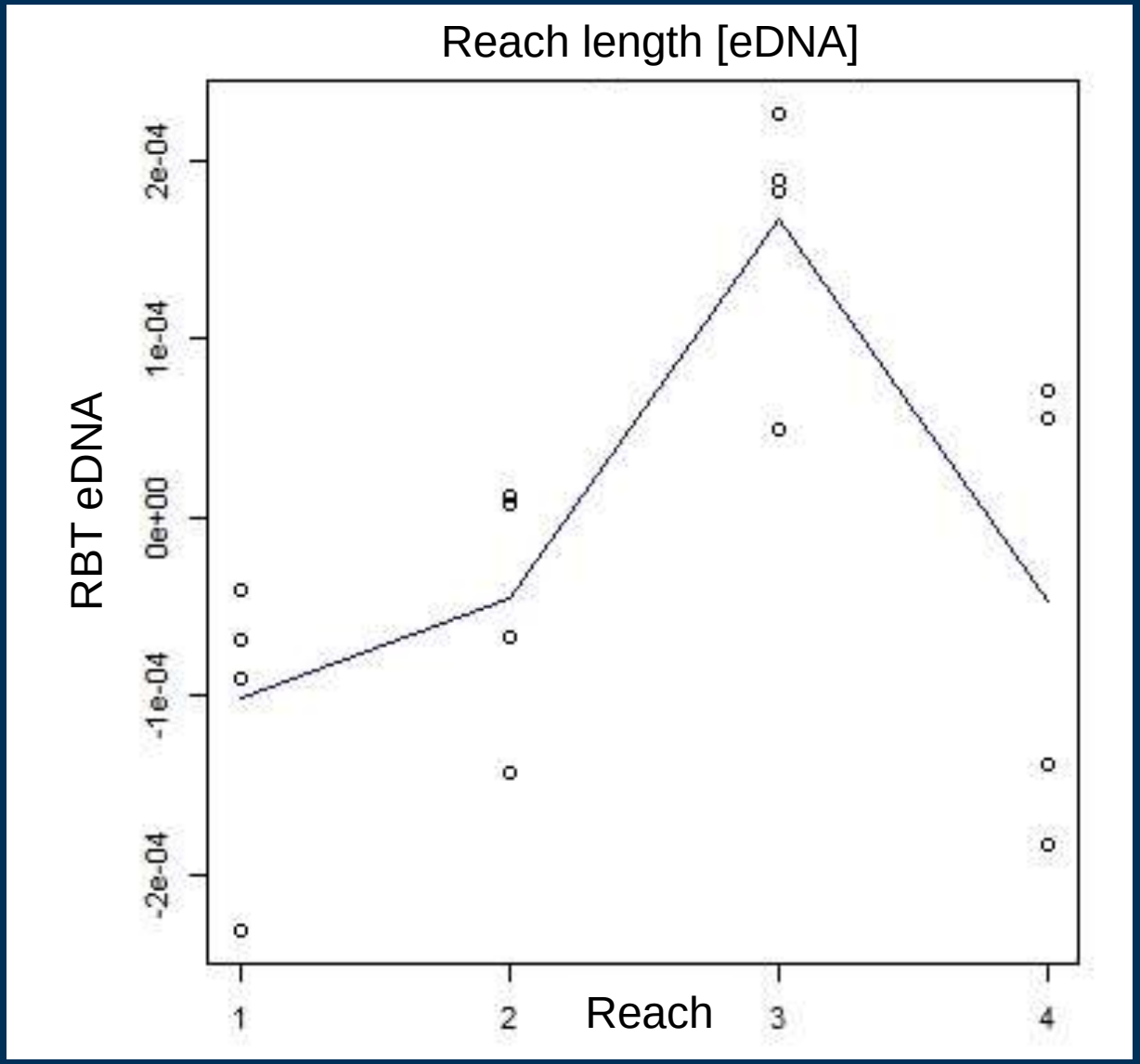
eDNA as an index of relative abundance



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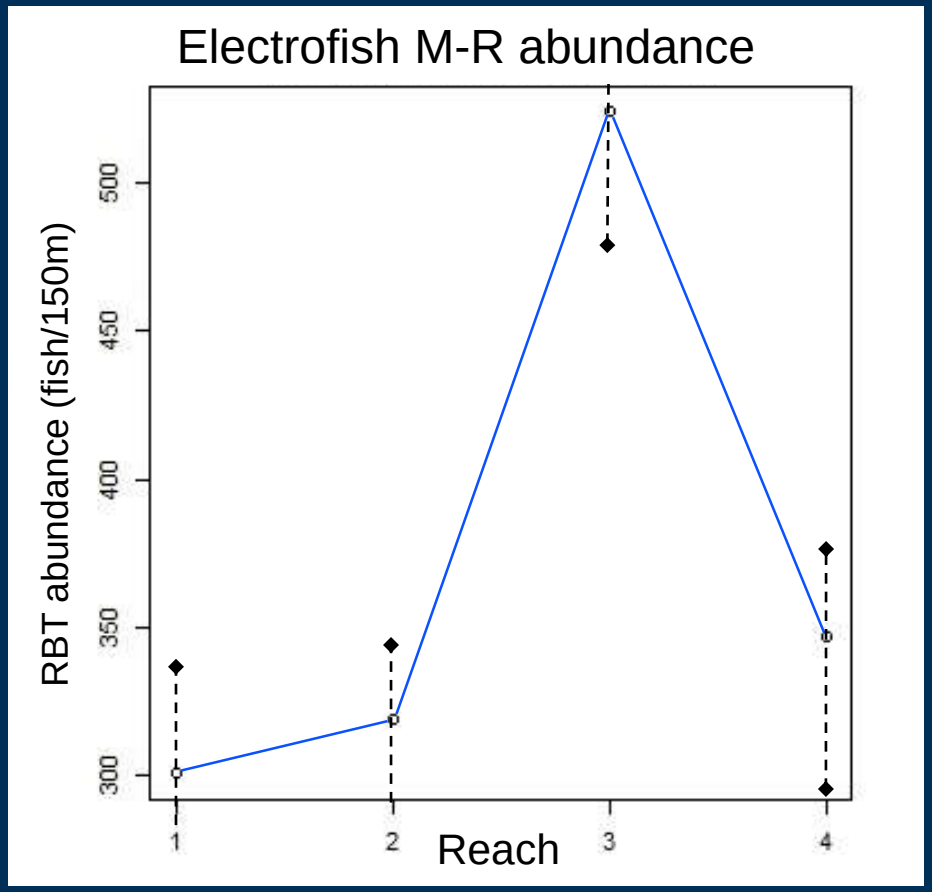
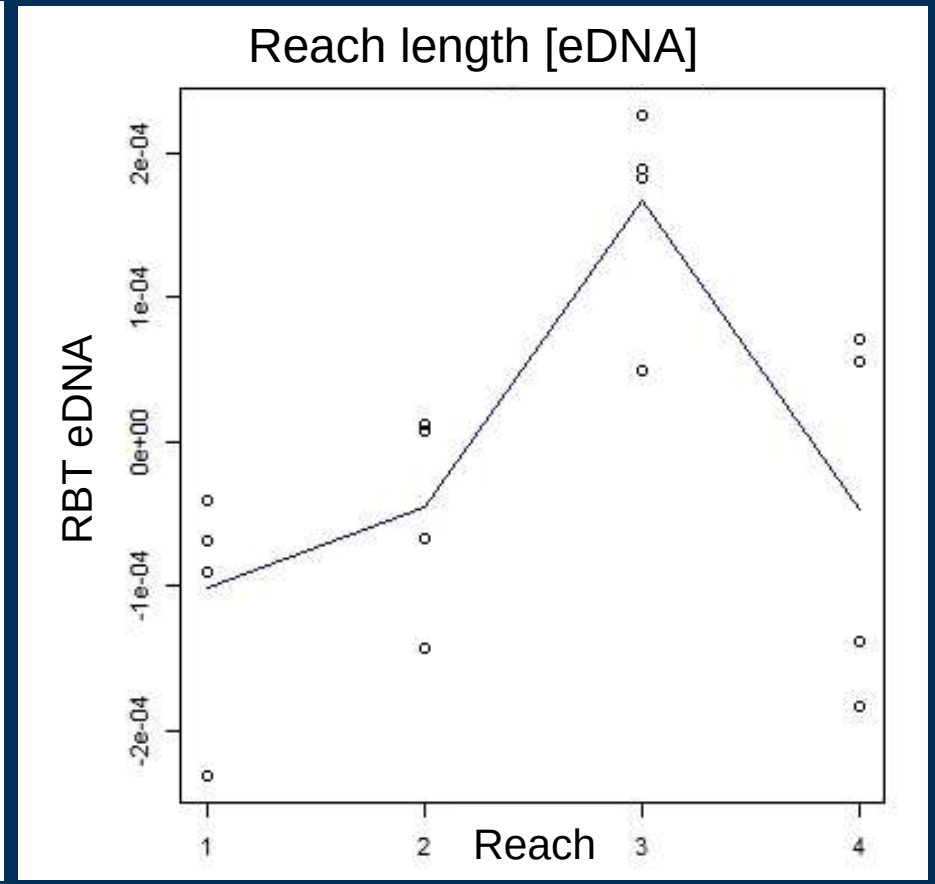
eDNA as an index of relative abundance





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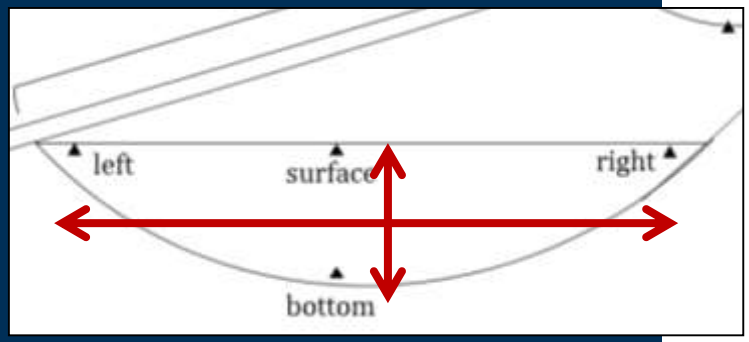
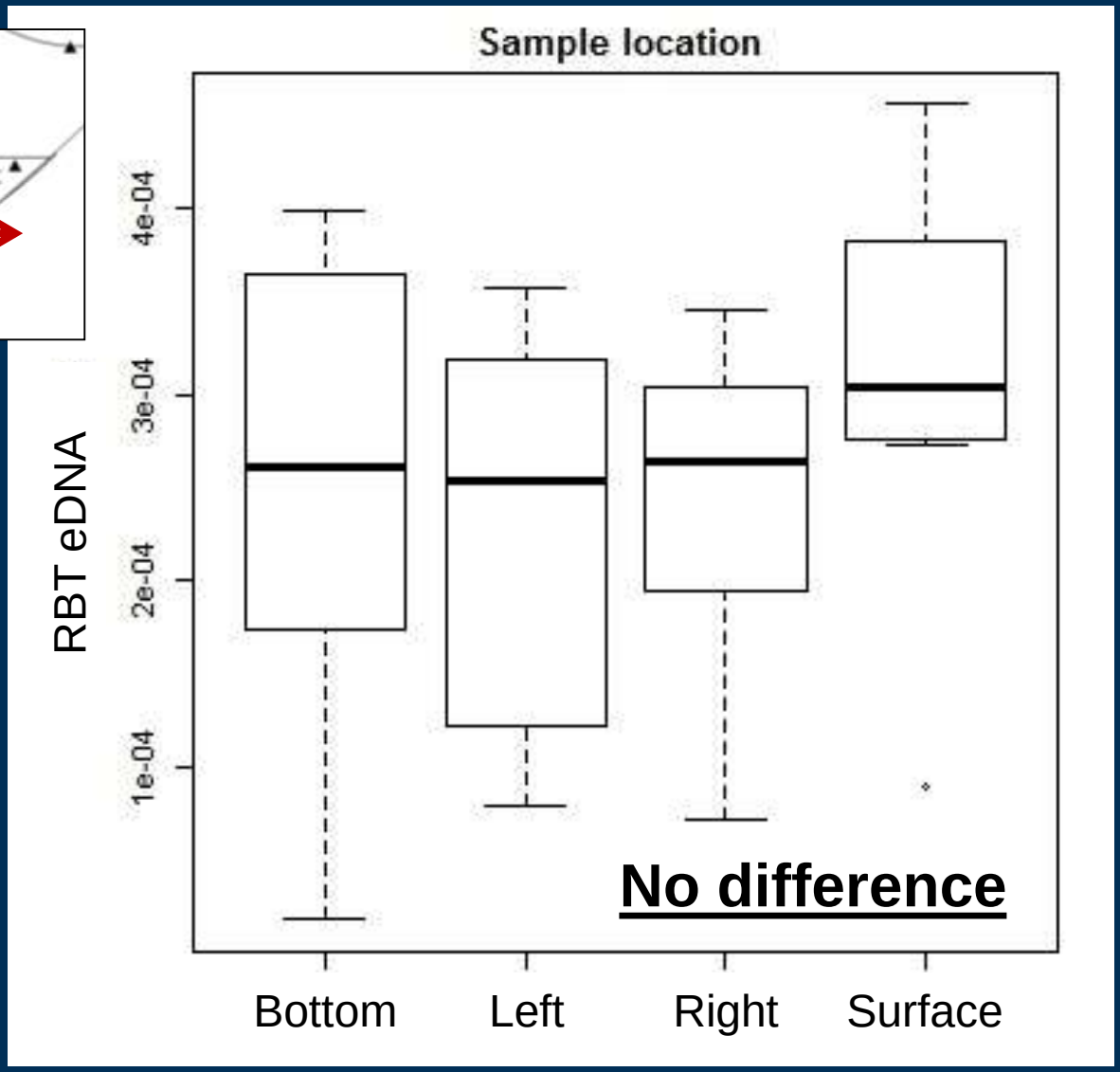
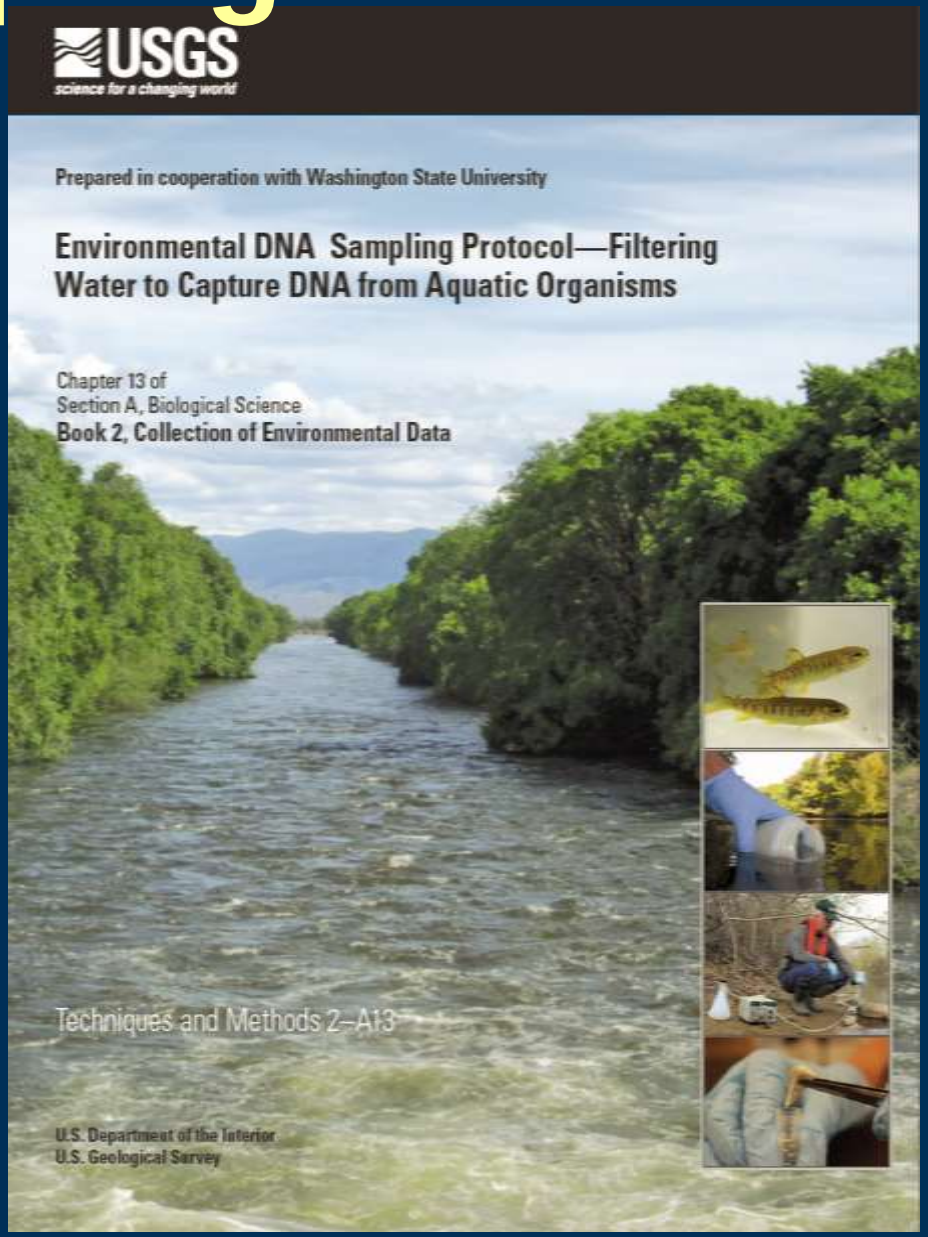
eDNA within the stream cross-section





eDNA Sampling Protocols

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



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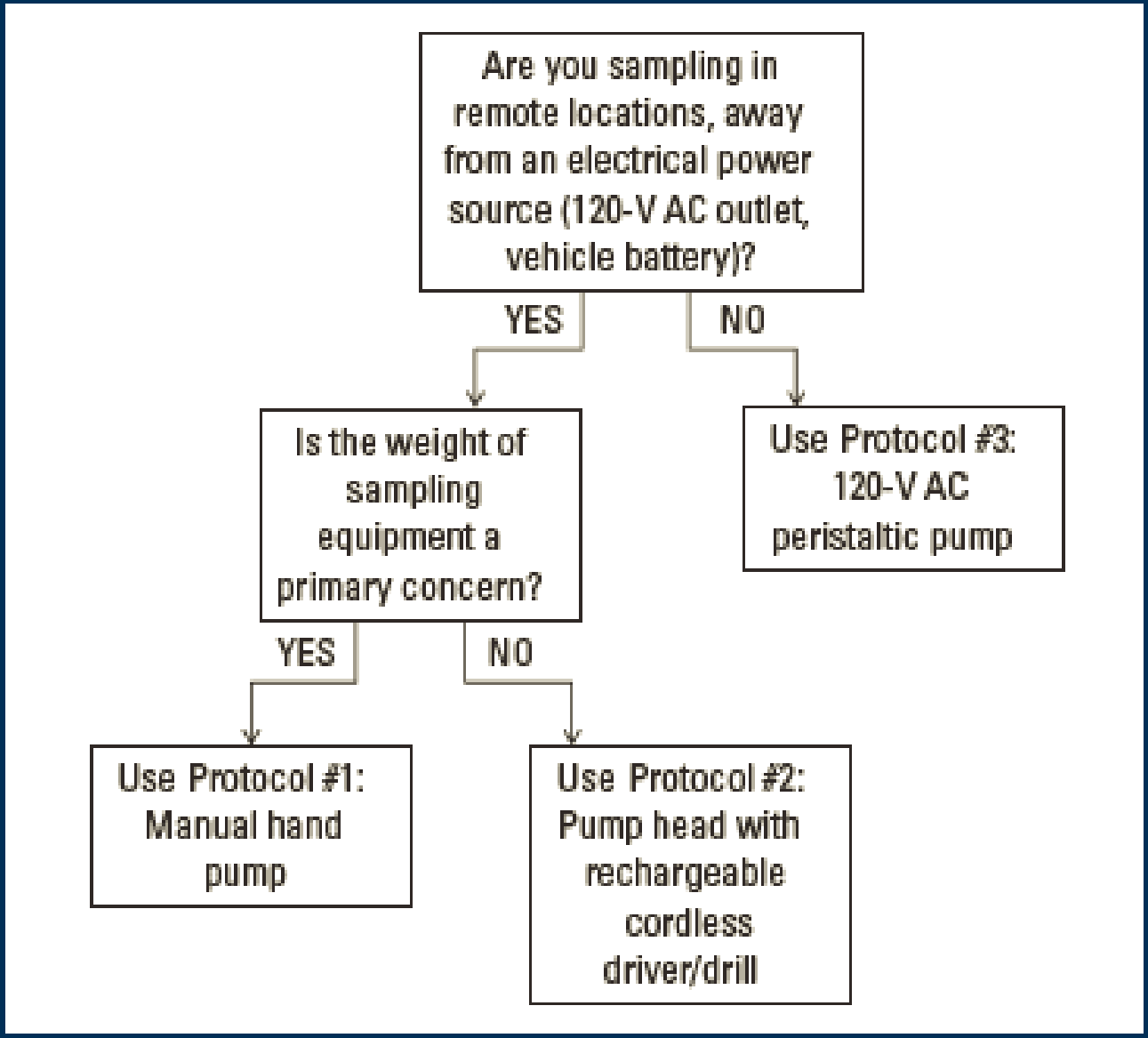
Selecting the best protocol



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Sampling Workflow Diagram

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

Or

Collect and pour

Step 3: Preserve water sample

Ethanol-filled vial

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Protocol #1: Hand pump

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Protocol #2: Cordless driver

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Protocol #3: 120v pump

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Sample collection options

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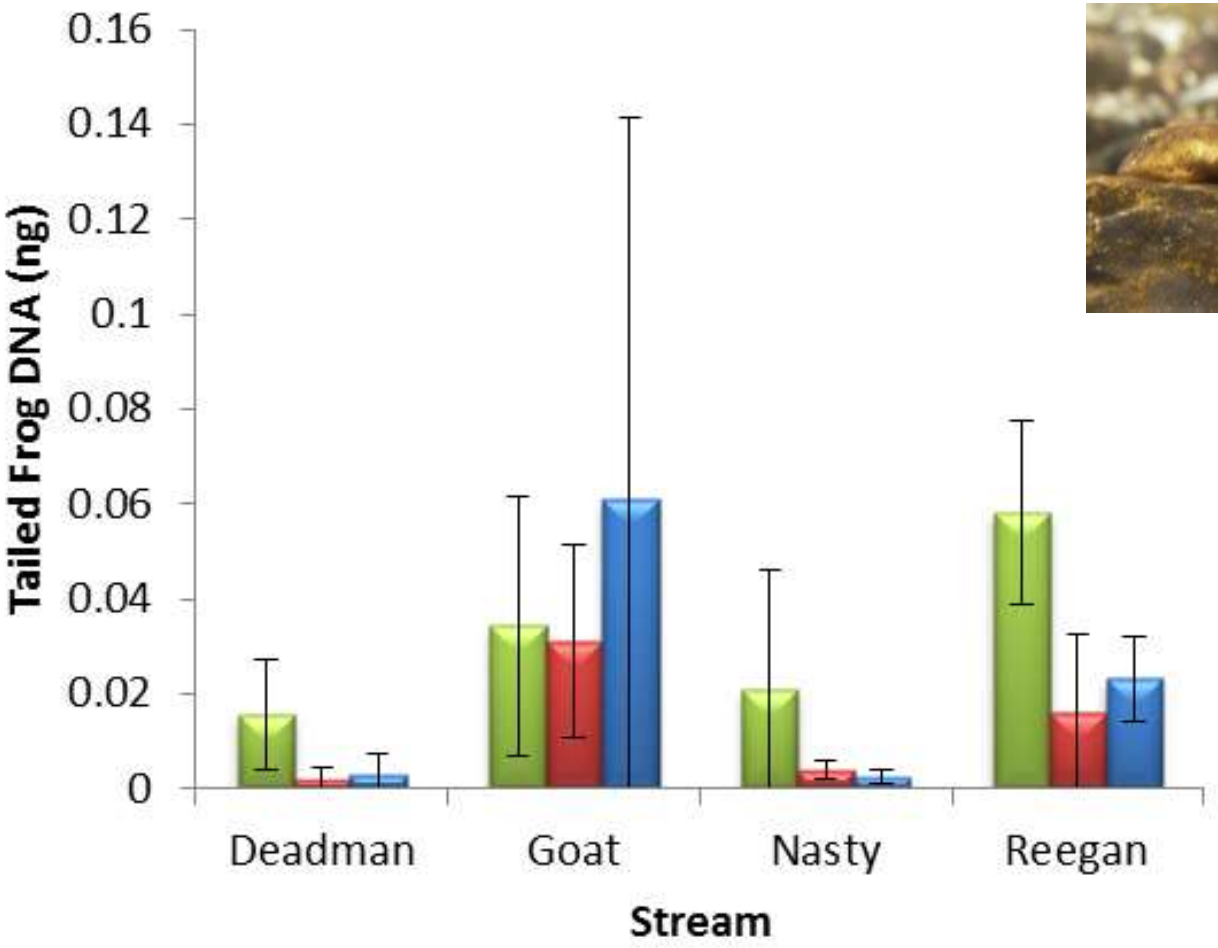
Comparing sample collection options



P = 0.02 Instream samples higher

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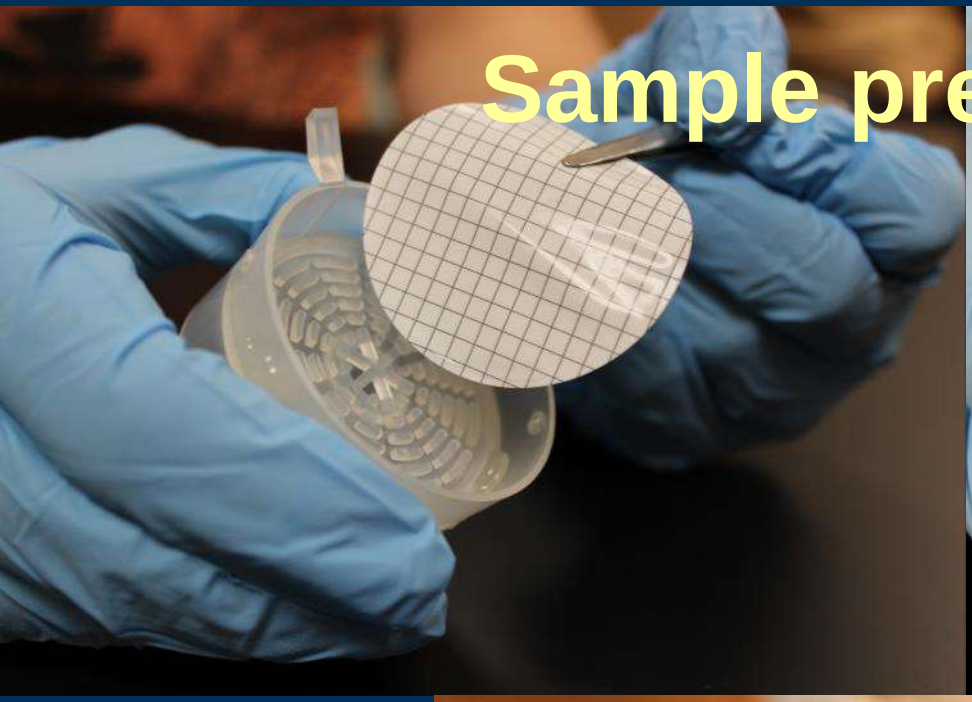
Pilliod et al. (2013). Canadian Journal Fisheries and Aquatic Sciences.

Sample preservation



Filters stored in ethanol at room temp

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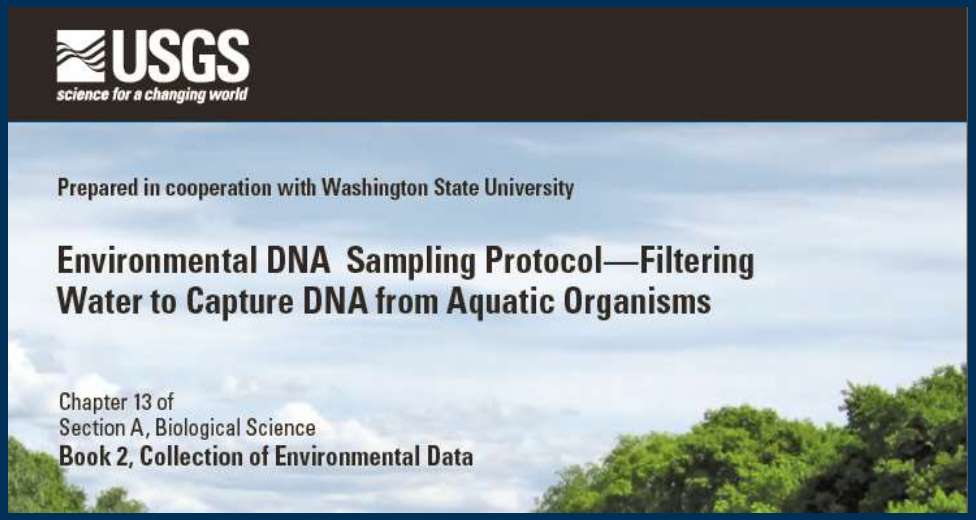
eDNA Resources



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

USGS FACT SHEET
http://pubs.usgs.gov/fs/2012/3146/pdf/fs2012-3146.pdf

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eDNA Resources

eDNA.fisheries.org

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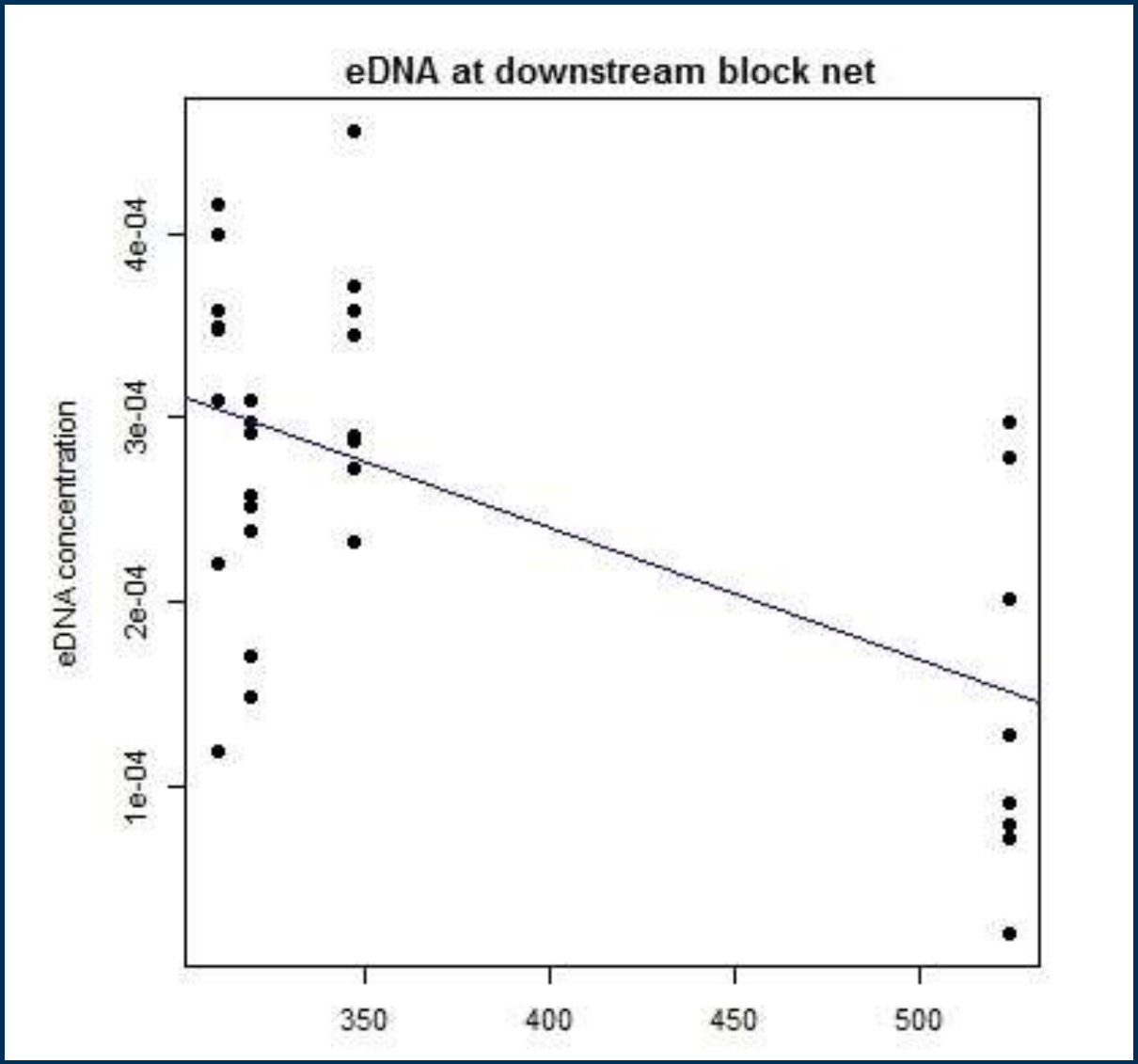
Photo courtesy of Jeffrey Williams (AKDFG)

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


eDNA at downstream block net
eDNA concentration
1e-04
2e-04
3e-04
4e-04
350
400
450
500

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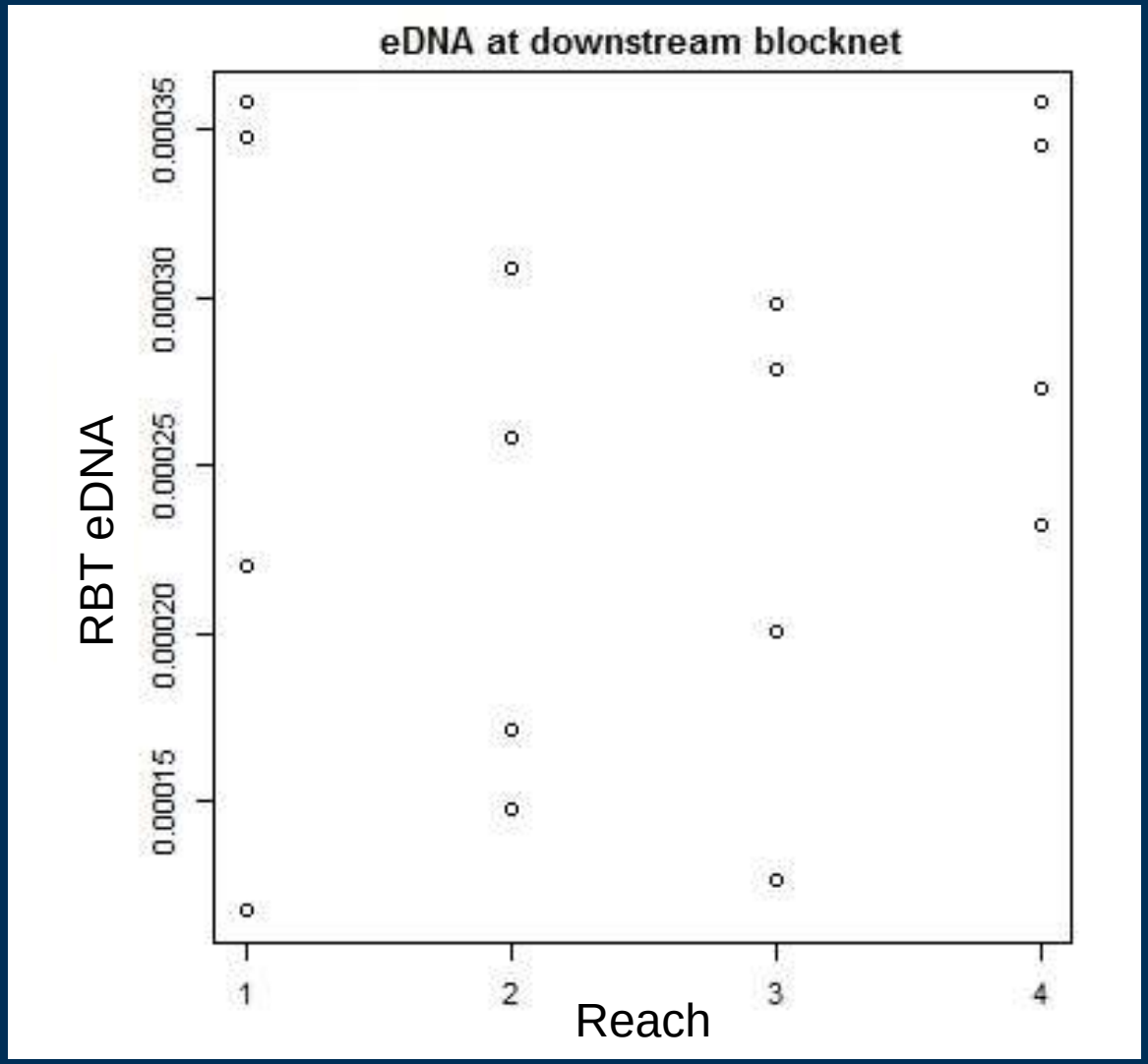
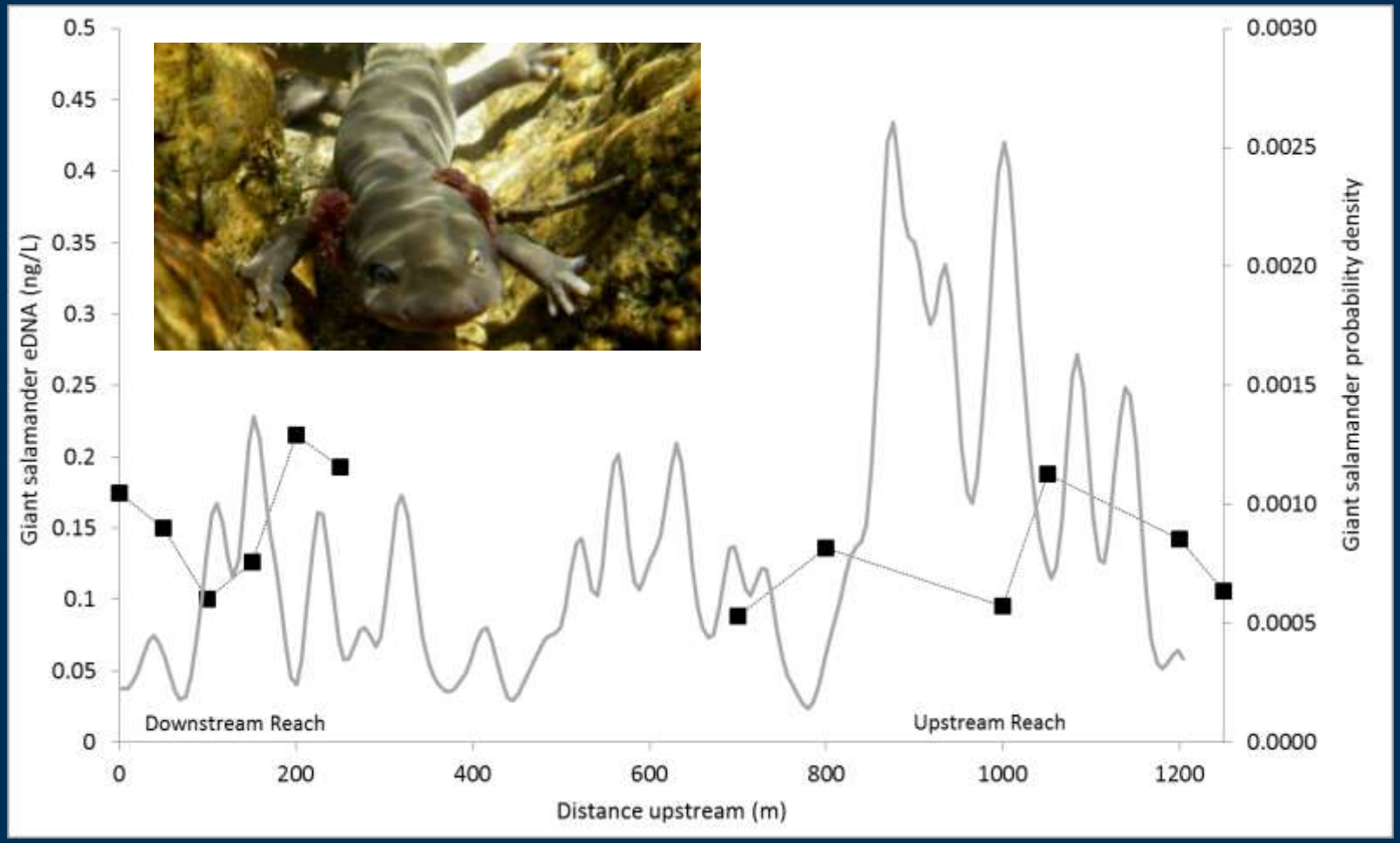

eDNA at downstream blocknet
RBT eDNA
0.00015
0.00020
0.00025
0.00030
0.00035
1
2
3
4
Reach

What does an eDNA sample represent?



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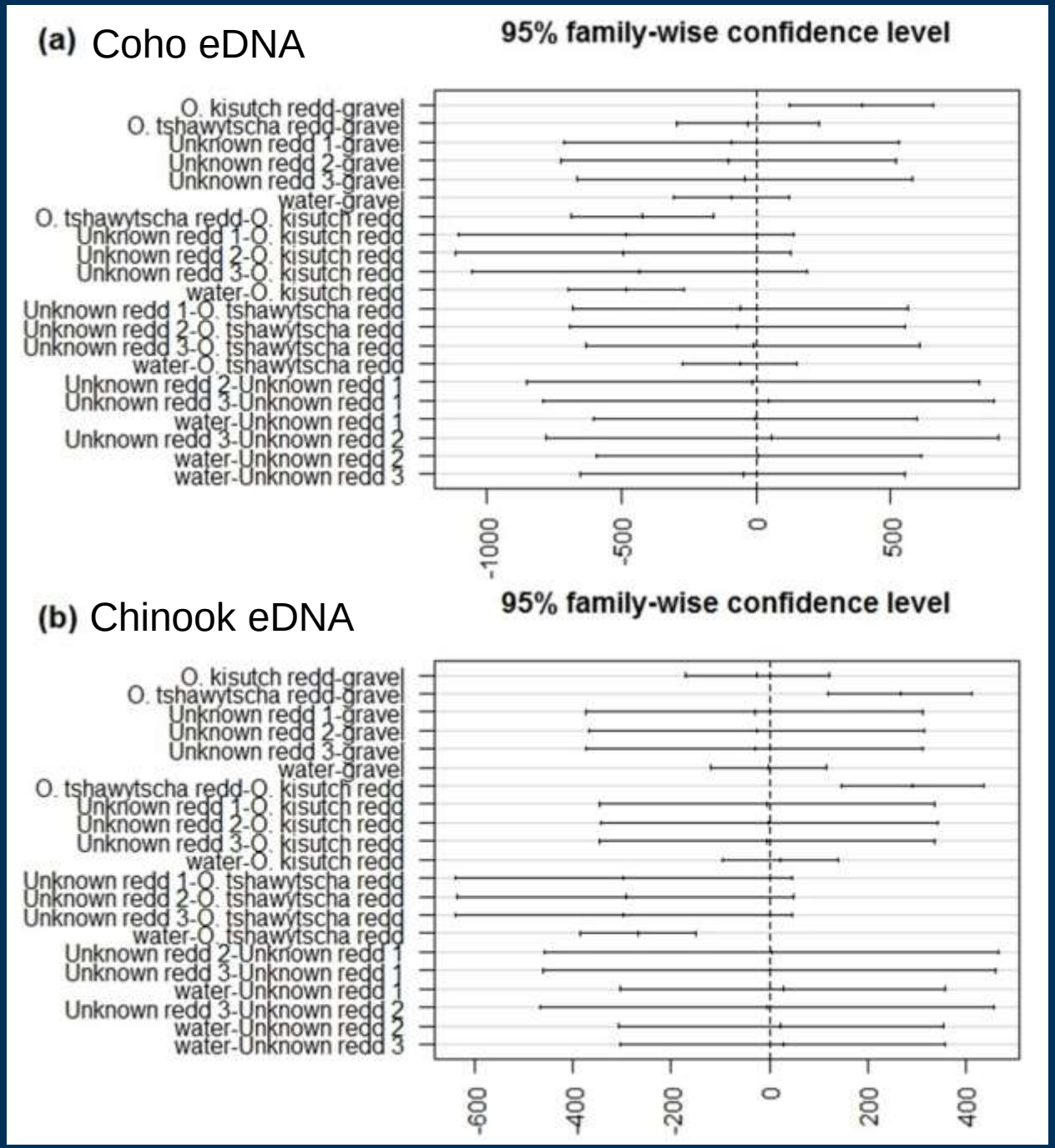
Pilliod et al. (2013) Canadian Journal of Fisheries and Aquatic Sciences 70:1123-1130.


(a) Coho eDNA
95% family-wise confidence level
O. kisutch redd-gravel
O. tshawytscha redd-gravel
Unknown redd 1-gravel
Unknown redd 2-gravel
Unknown redd 3-gravel
water-gravel
O. tshawytscha redd-O. kisutch redd
Unknown redd 1-O. kisutch redd
Unknown redd 2-O. kisutch redd
Unknown redd 3-O. kisutch redd
water-O. kisutch redd
Unknown redd 1-O. tshawytscha redd
Unknown redd 2-O. tshawytscha redd
Unknown redd 3-O. tshawytscha redd
water-O. tshawytscha redd
Unknown redd 2-Unknown redd 1
Unknown redd 3-Unknown redd 1
water-Unknown redd 1
Unknown redd 3-Unknown redd 2
water-Unknown redd 2
water-Unknown redd 3
-1000
-500
0
500
(b) Chinook eDNA
95% family-wise confidence level
O. kisutch redd-gravel
O. tshawytscha redd-gravel
Unknown redd 1-gravel
Unknown redd 2-gravel
Unknown redd 3-gravel
water-gravel
O. tshawytscha redd-O. kisutch redd
Unknown redd 1-O. kisutch redd
Unknown redd 2-O. kisutch redd
Unknown redd 3-O. kisutch redd
water-O. kisutch redd
Unknown redd 1-O. tshawytscha redd
Unknown redd 2-O. tshawytscha redd
Unknown redd 3-O. tshawytscha redd
water-O. tshawytscha redd
Unknown redd 2-Unknown redd 1
Unknown redd 3-Unknown redd 1
water-Unknown redd 1
Unknown redd 3-Unknown redd 2
water-Unknown redd 2
water-Unknown redd 3
-600
-400
-200
0
200
400

USGS

Table 3. Analysis of Variance (ANOVA) table for differences in O. kisutch eDNA among sample types.						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Sample type	6	1847497	307916	8.1409	1.997e-06 ***	
Residuals	59	2231569	37823			
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.				
Sample type	diff	lwr	upr	p adj
O. tshawytscha redd - gravel	266.7449	120.5793	412.91037	0.0000133
O. tshawytscha redd - O. kisutch redd	291.8099	145.6444	437.97538	0.0000018
Water - O. tshawytscha redd	-269.2079	-387.1876	-151.22823	0.0000001
Fit: aov(formula = Site replicate mean [eDNA] (pg/15mL)~ Sample type, data = O. tshawytscha eDNA)				

USGS

How many replicates are necessary?

Possible detection outcomes at a site	% of sites	# replicates (-)	# replicates total
0 0 0	41%		
1 0 0	3%	6	9
1 1 0	7%	6	18
1 1 1	49%	0	135
Total	100%	12	162

USGS

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

Assessing detection probability and error

Possible detection outcomes at a site	% of sites	# replicates (-)	# replicates total
0 0 0	41%		
1 0 0	3%	6	9
1 1 0	7%	6	18
1 1 1	49%	0	135
Total	100%	12	162

12/162 = **7% false negatives**

USGS

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

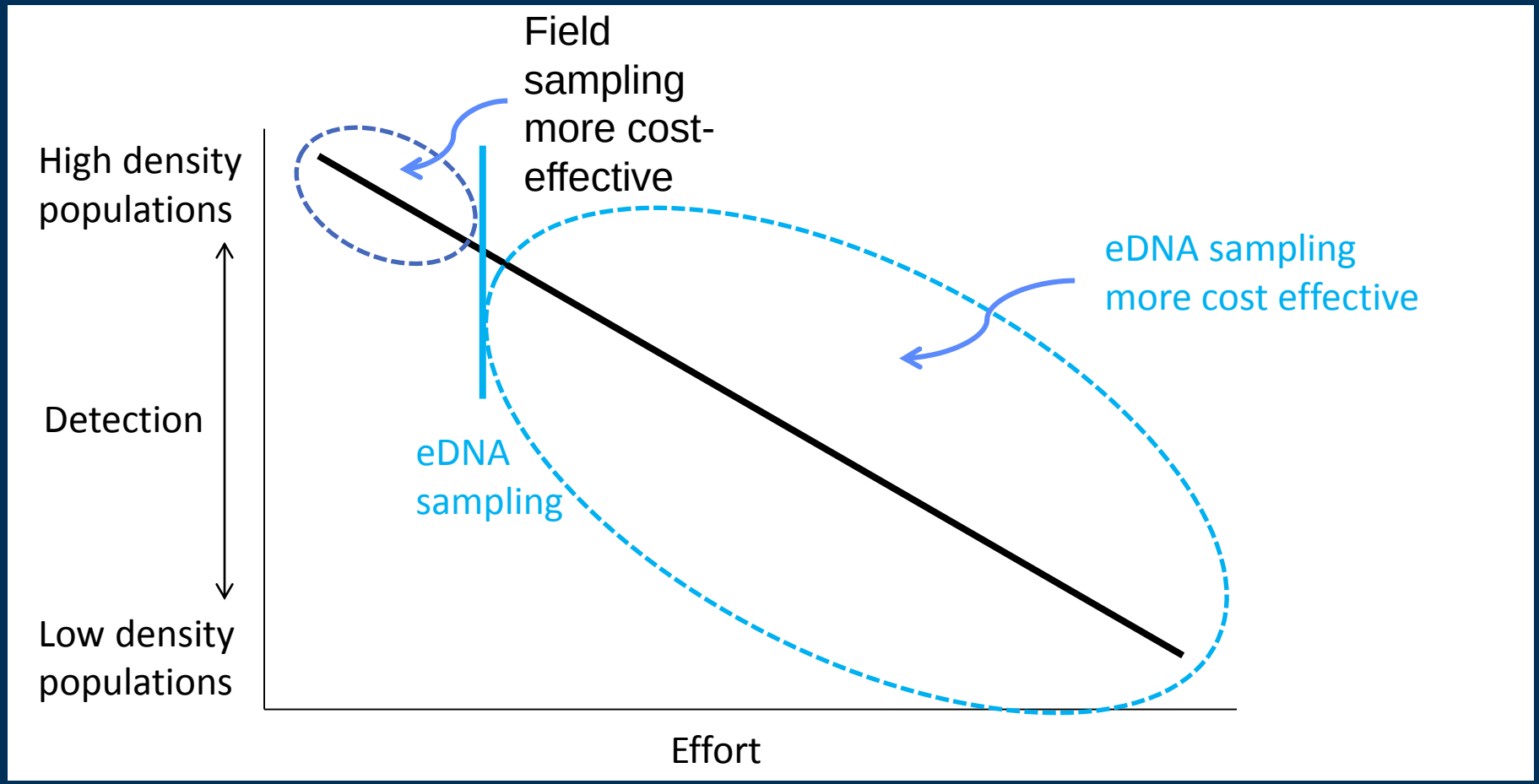
Contamination Prevention

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.

USGS

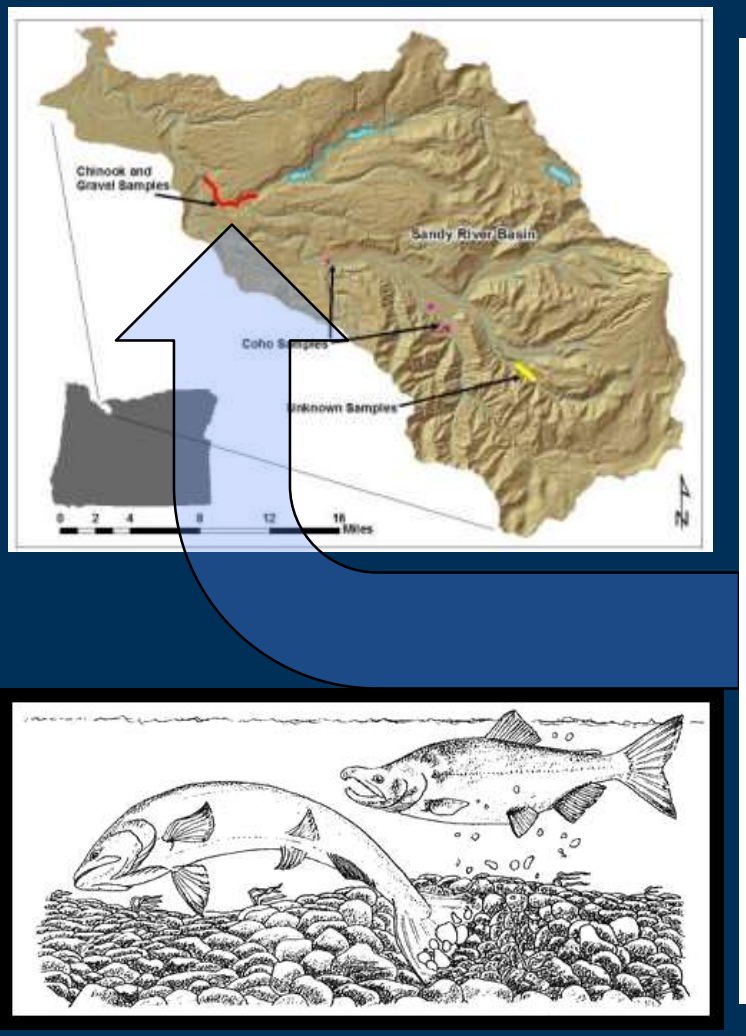
When do we use eDNA?

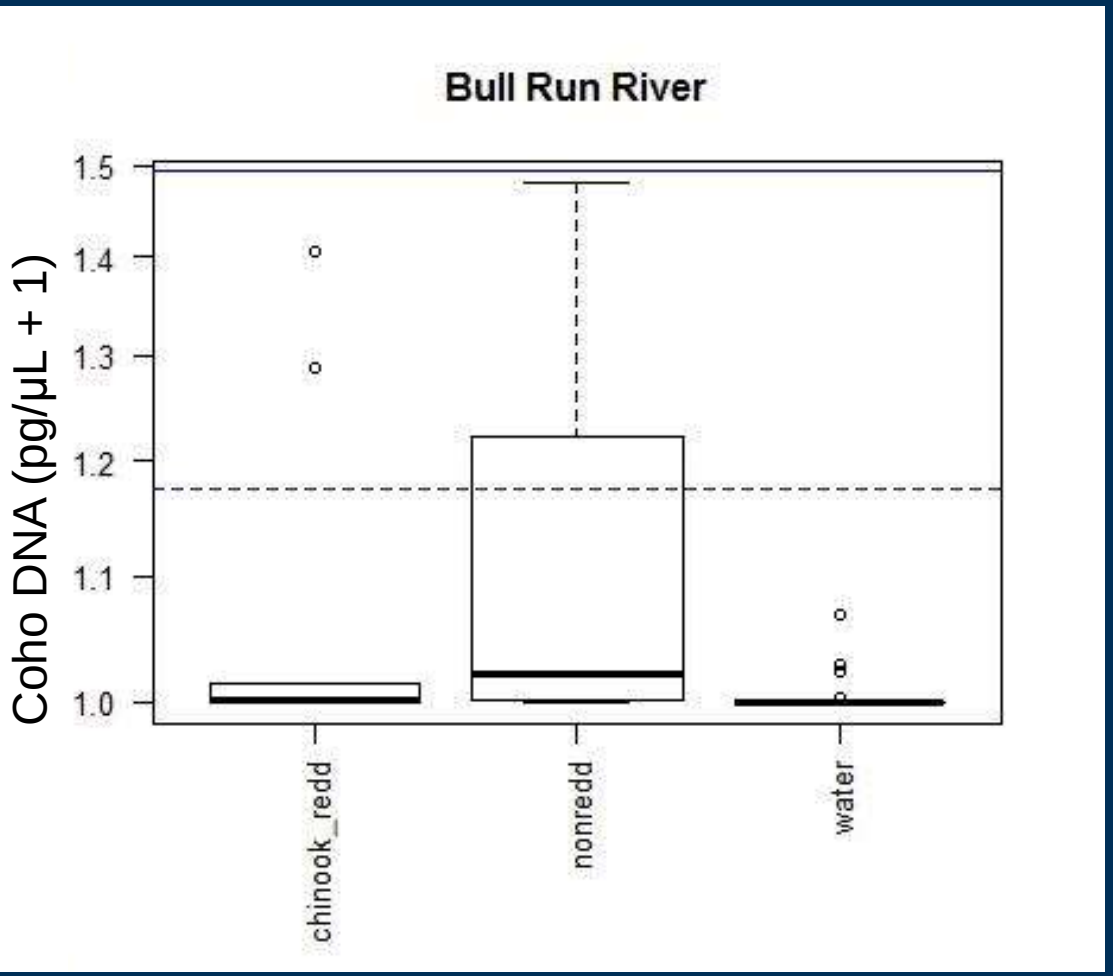


USGS

Figure courtesy of Dr. Caren Goldberg, WSU

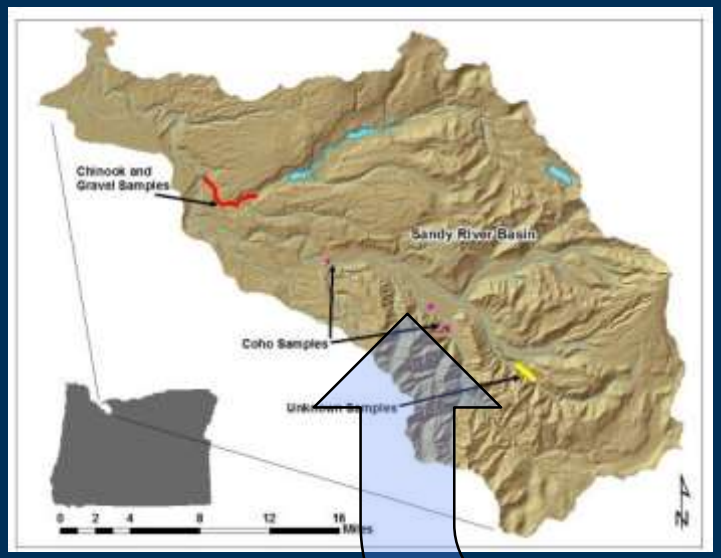
How much Coho DNA is in environment?

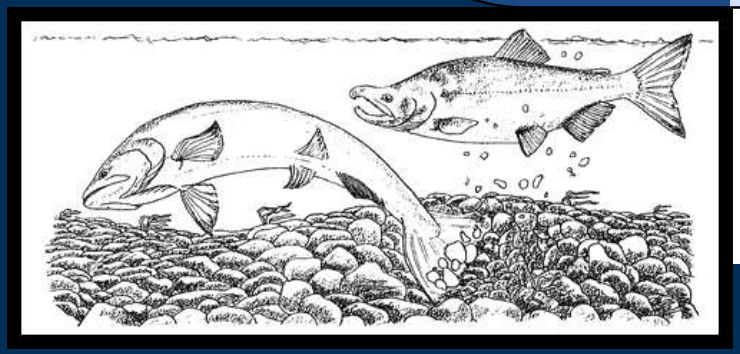


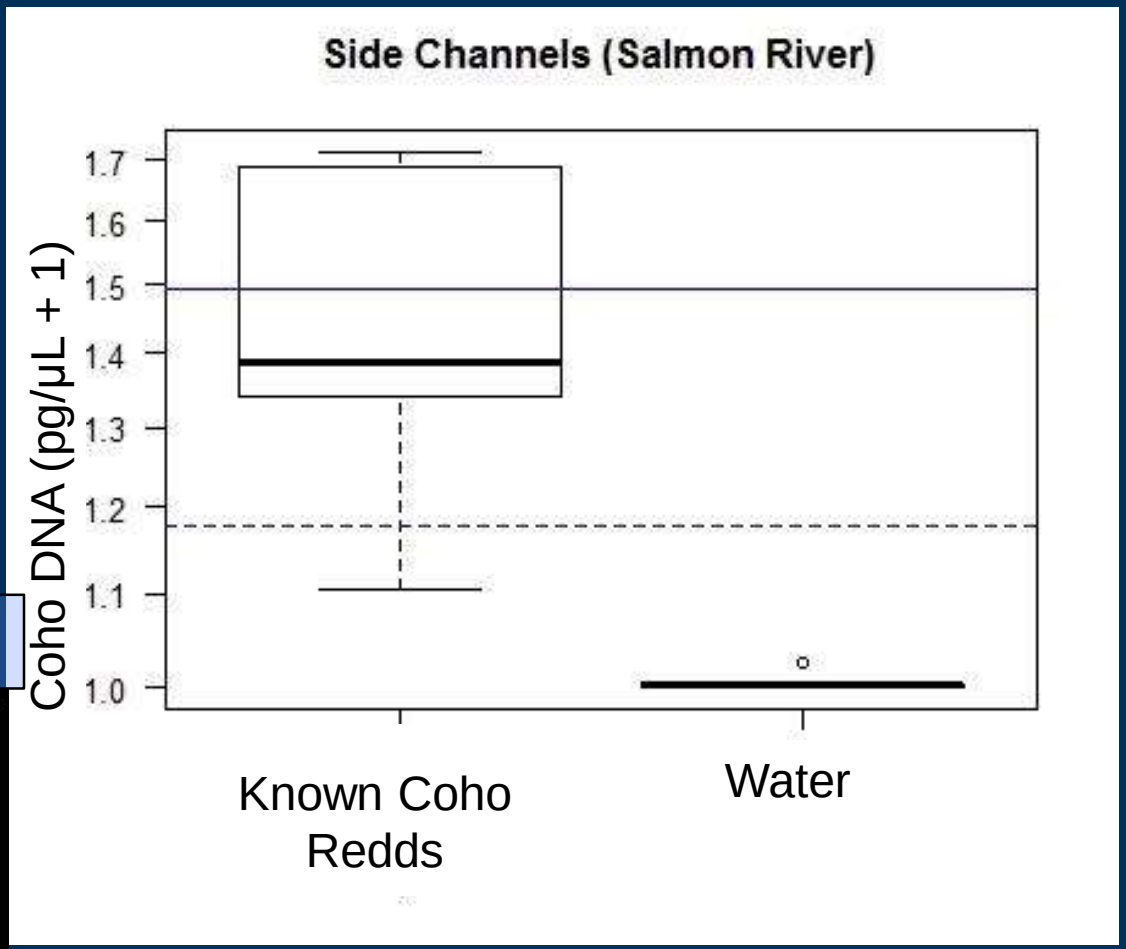


USGS

How much Coho DNA is at a Coho Redd?







USGS