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## Using eDNA to Understand Changes in Aquatic Biodiversity Above and Below a Barrier

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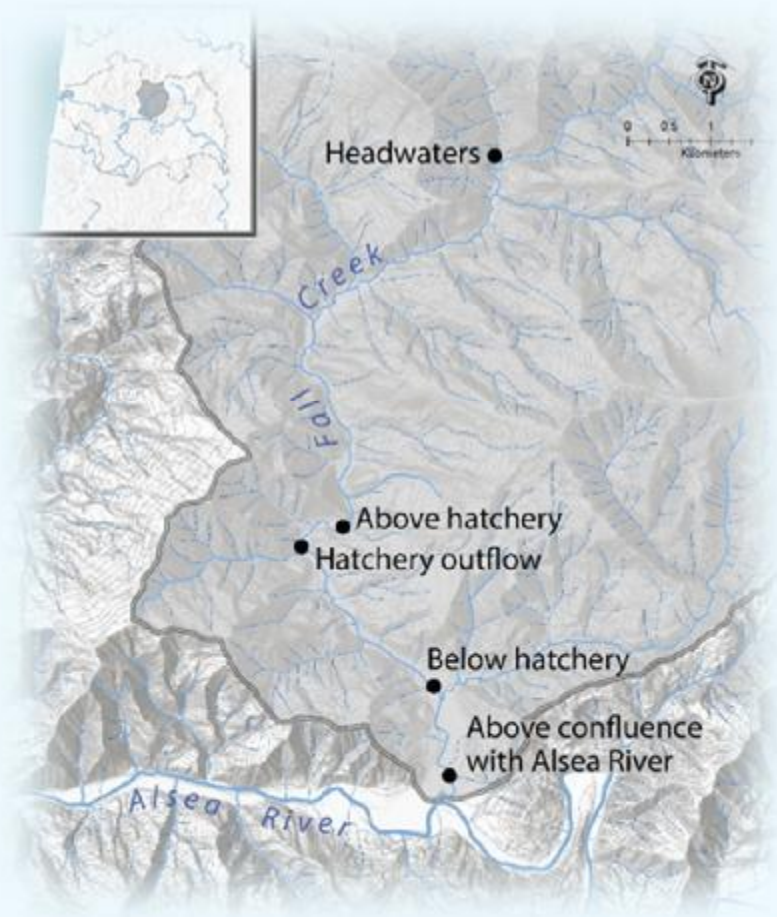
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# Using eDNA to Understand Changes in Aquatic Biodiversity Above and Below a Barrier



Brooke Penaluna, Rich Cronn,  
and Laura Hauck  
PNW Research Station  
US Forest Service



# Acknowledgements

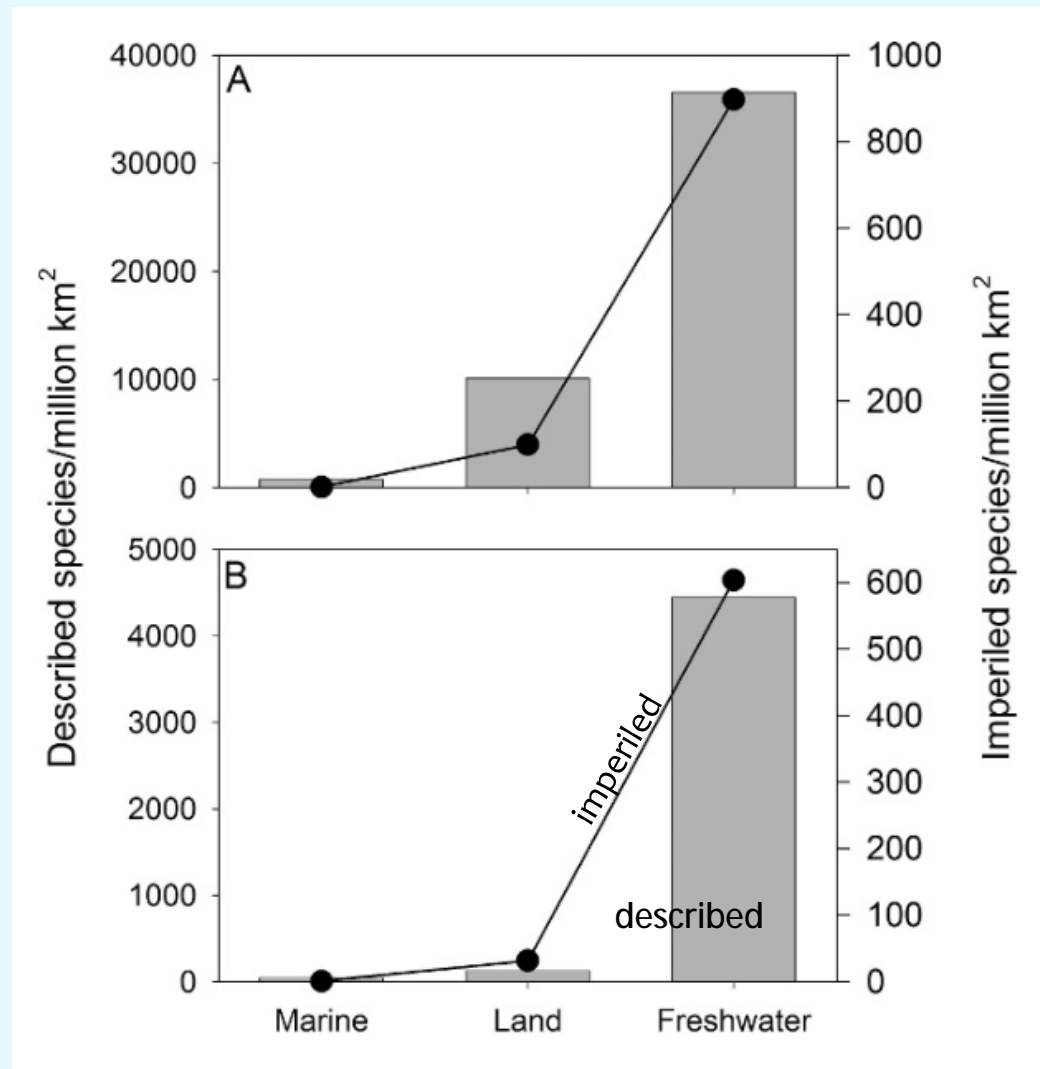
- Partners: Aquatic and Riparian Effectiveness Monitoring Program, Olympic Experimental State Forest
- Funding Partners: NCASI, Weyerhaeuser, BLM, PNW Research Station
- Bob Bilby, Bob Danehy, Stephanie Miller, Jessica Homyack, Erik Schilling, Jake Verschuyt, Chris Hirsch, Kathryn Ronnenberg, Kelly Christiansen, Mark Raggon, Bruce Hansen, Loretta Ellenberg, Becky Flitcroft, Teddy Minkova, Kyle Martens, Sky Croppers

**ncasi**

National Council for Air  
and Stream Improvement



# Worldwide freshwater biodiversity is among most imperiled



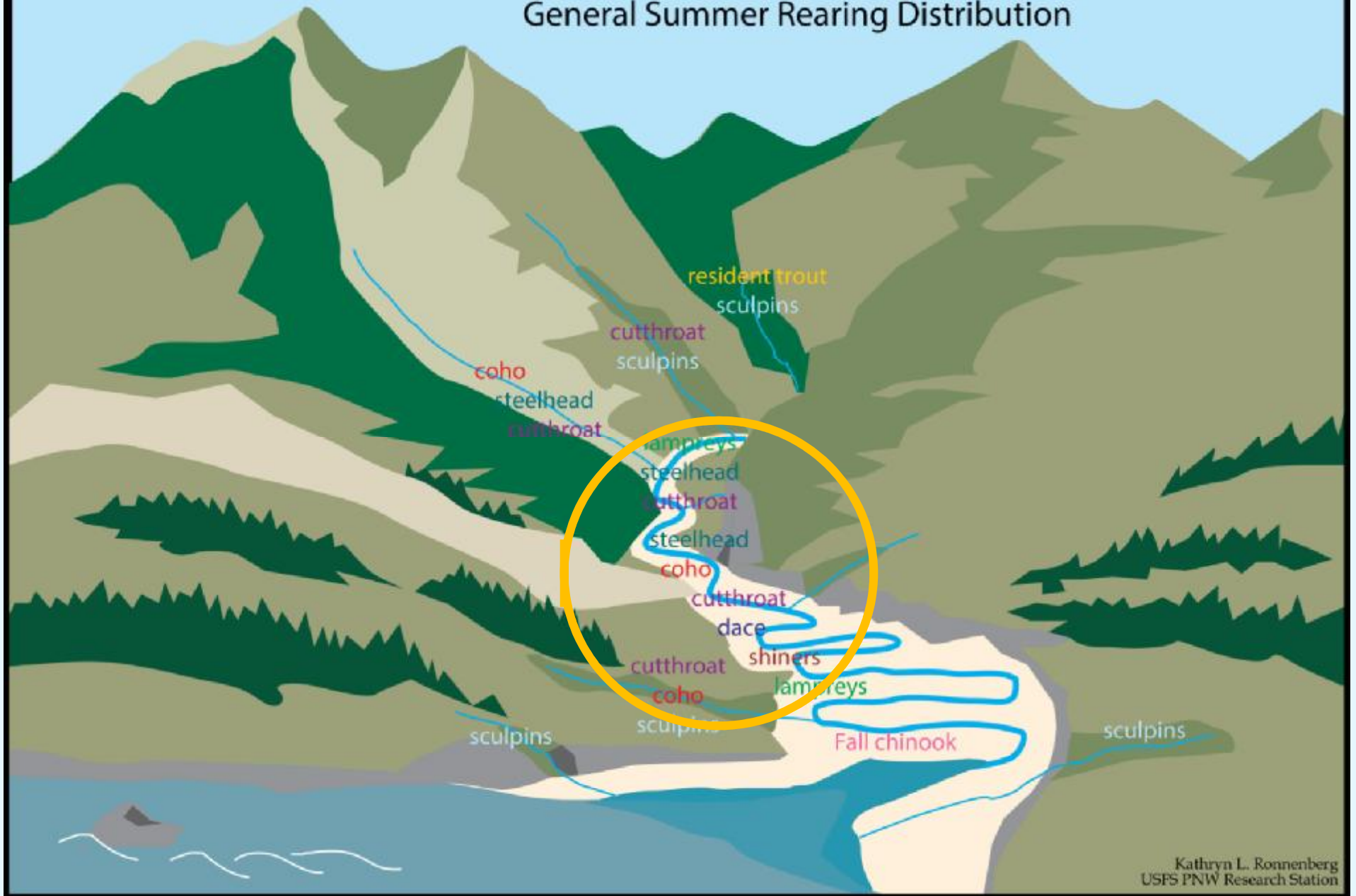
Strayer and Dudgeon 2010

# Traditional sampling for aquatic species

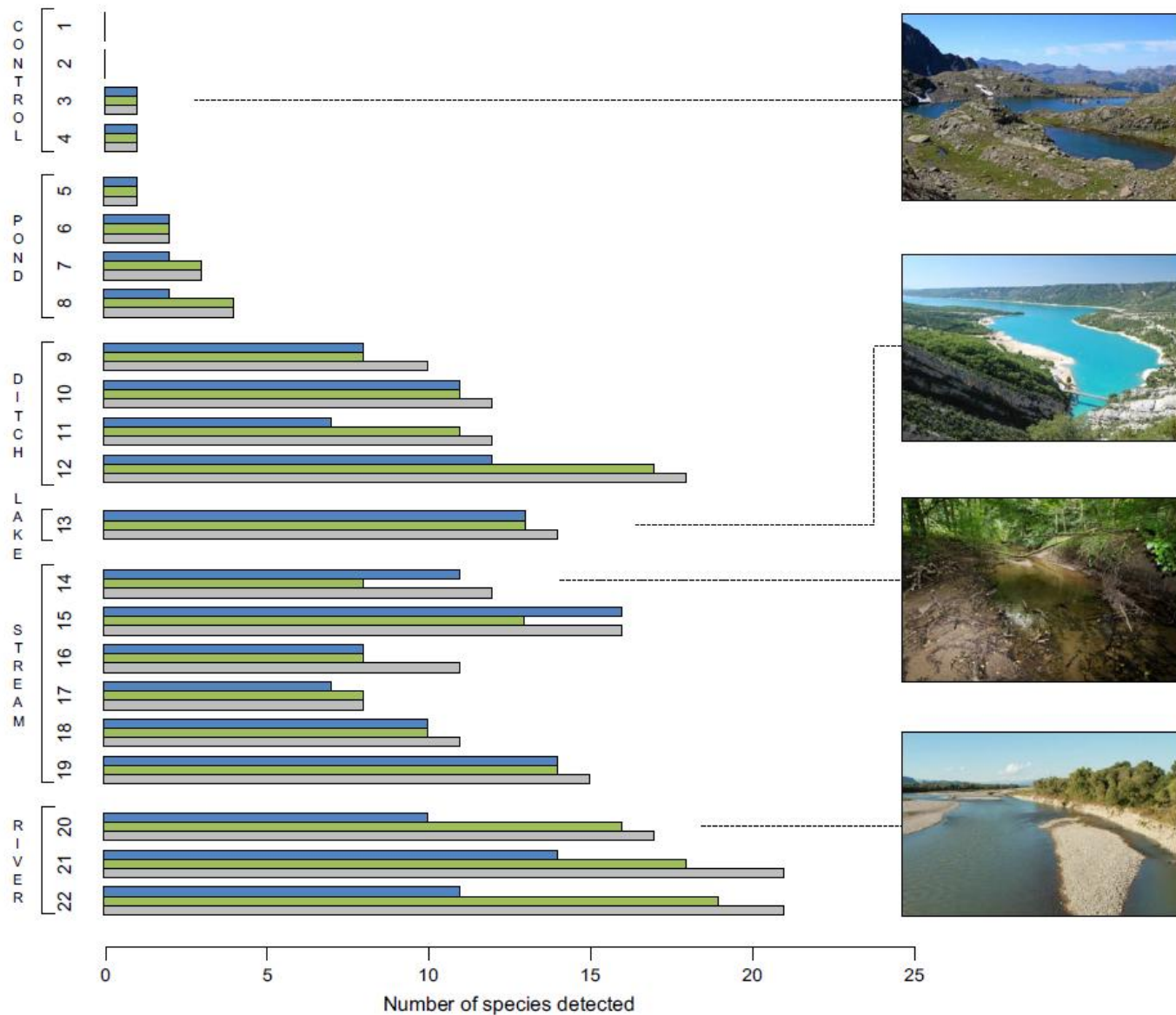
- Based on visual detections and counting, which is not always standardized and is dependent on practical and taxonomic expertise
- Often limited to assessments of adults, often of listed species
- Occurs in summer
- Limited understanding year-round and for all life stages





# General Summer Rearing Distribution




# More fish species detected using eDNA metabarcoding



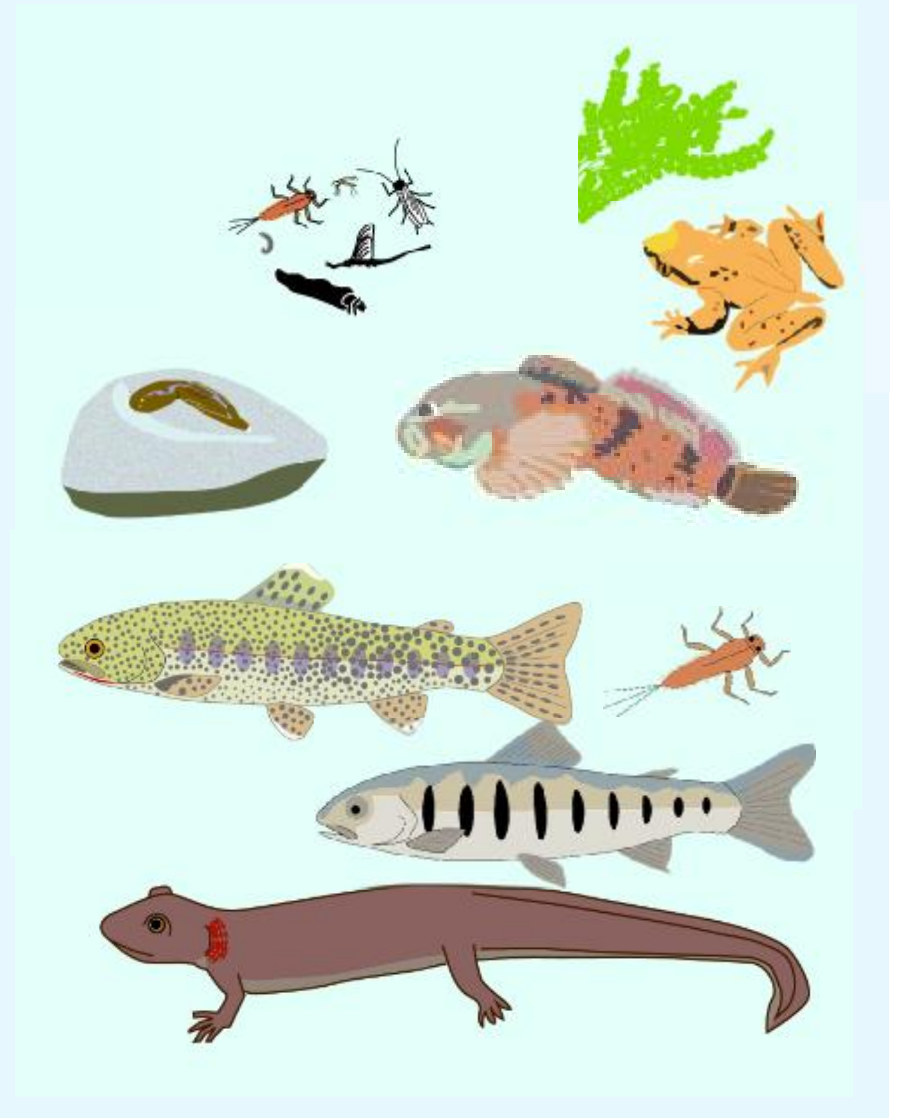
 Traditional surveys

 eDNA metabarcoding  
Using 1 gene (12S)

 Total fishes from both approaches

# Our goal: New Approach for obtaining species-specific aquatic data

- Rapid and accurate assessment
- Presence, abundance, and diversity of fish, amphibians, crayfish, and macroinvertebrates, especially during particular time periods or developmental stages
- Identification of common, endangered, rare and cryptic species





# eDNA Metabarcoding coupled with taxon-specific primers to identify several species from multiple taxon

Water Filtration



DNA Extraction



DNA Amplification  
of targets using PCR



48 targets x 48 samples  
= 2,304 PCR reactions

DNA Sequencing  
& Sequence Analysis



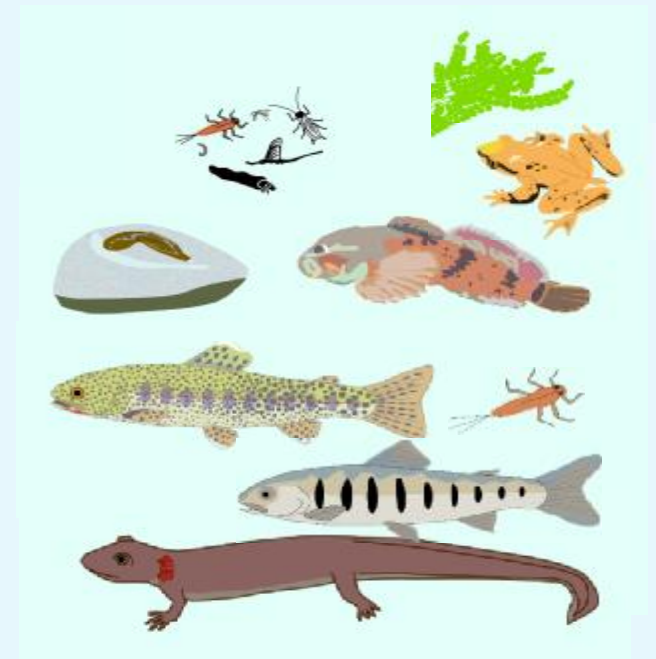
Stream Assemblage Profile  
Species detection and estimation of  
relative abundance of targets that  
were preferentially amplified

# Proof-of-concept Study on Fall Creek, OR

Can we equally detect multiple target species across diverse taxon?

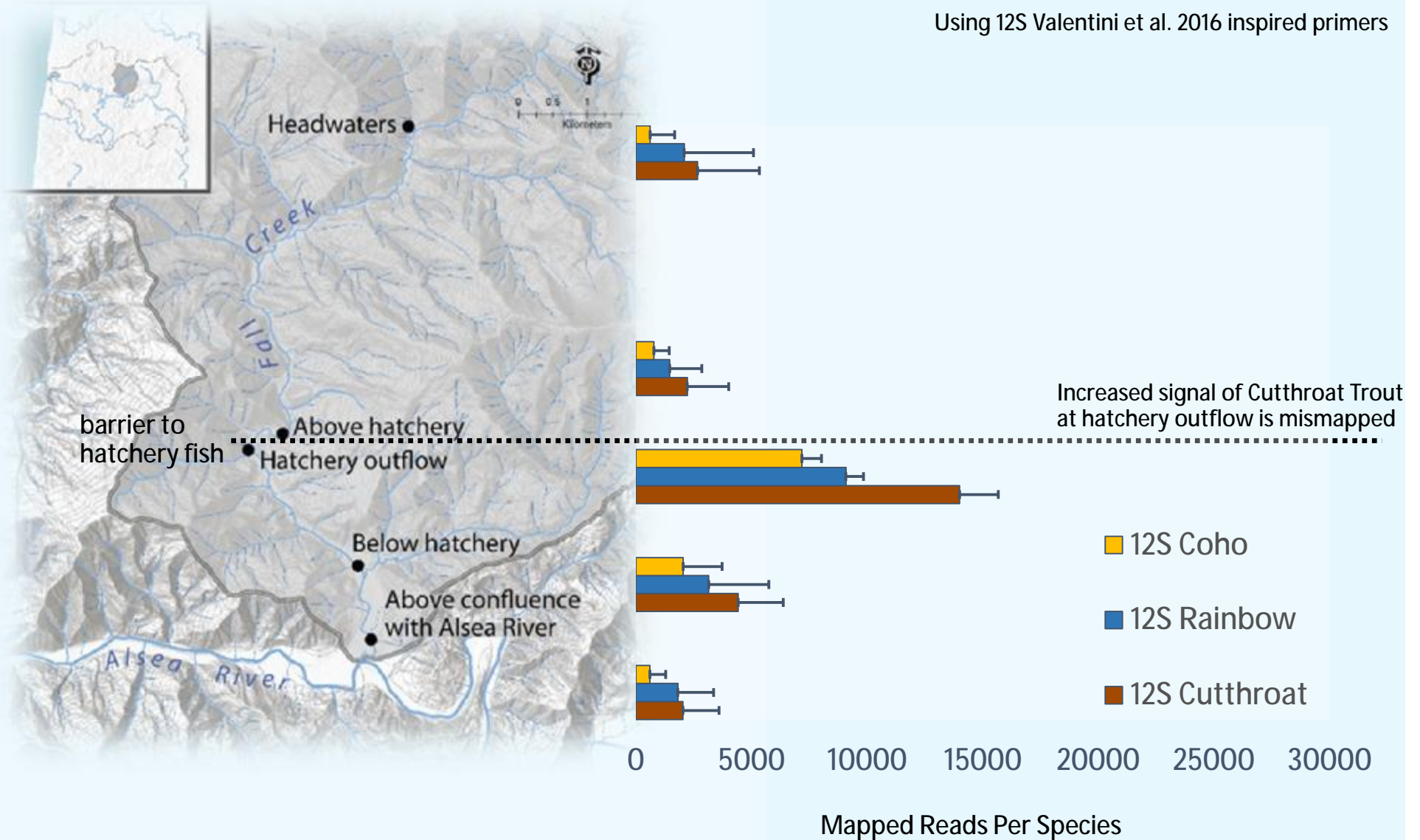
Common and rare species equally?

Does the aquatic assemblage change with a barrier?

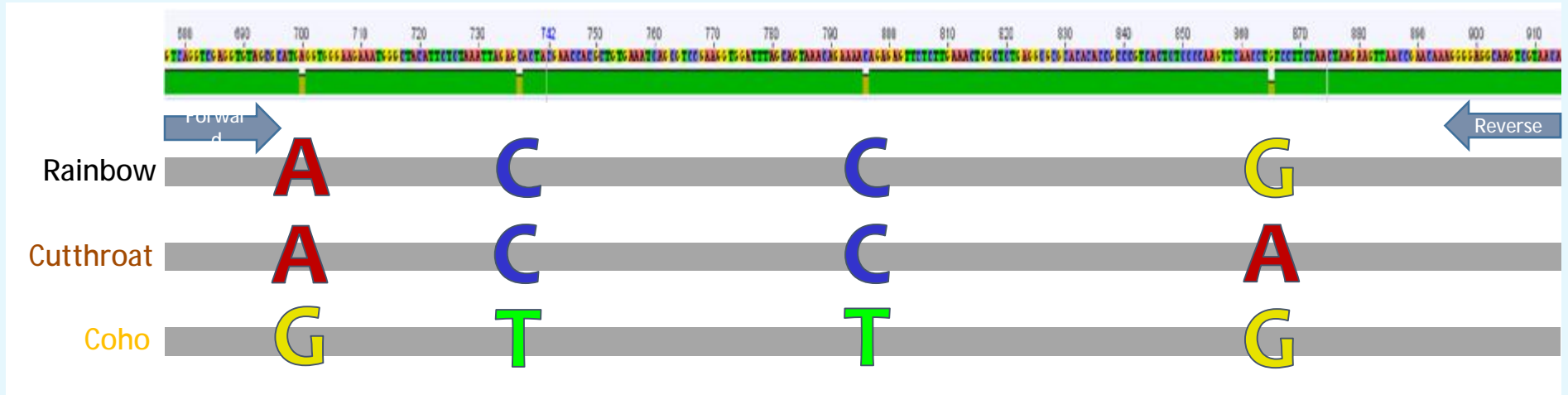


# Cutthroat Trout mismapped using taxon-general primer when Rainbow Trout numbers are high

Using 12S Valentini et al. 2016 inspired primers



# Salmonid 12S Universal Primer Alignment: 240 basepairs

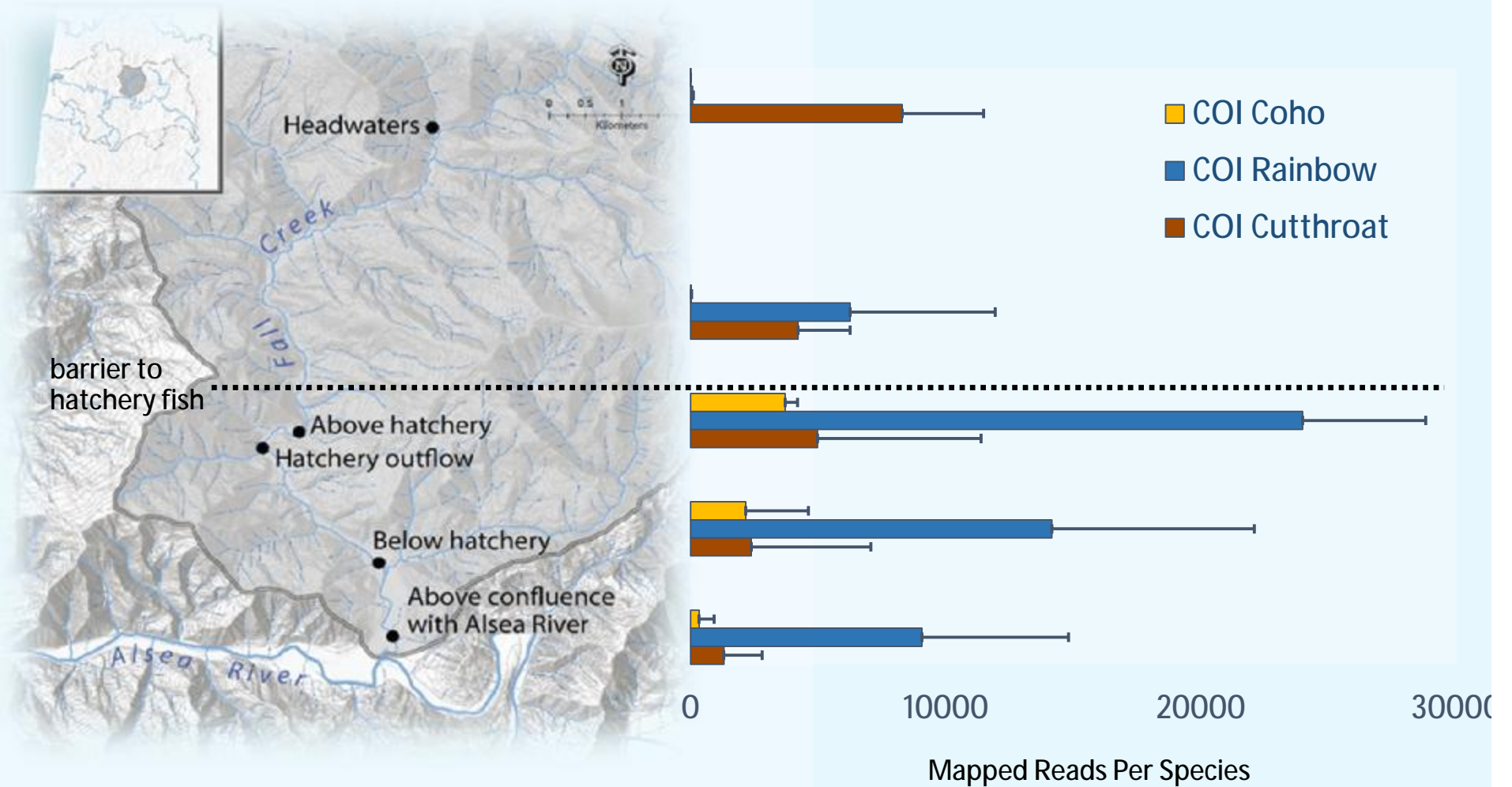


## Rainbow Trout: The Consensus Sequence

**Cutthroat:** 1/240 bp mismatch = 0.4% divergence

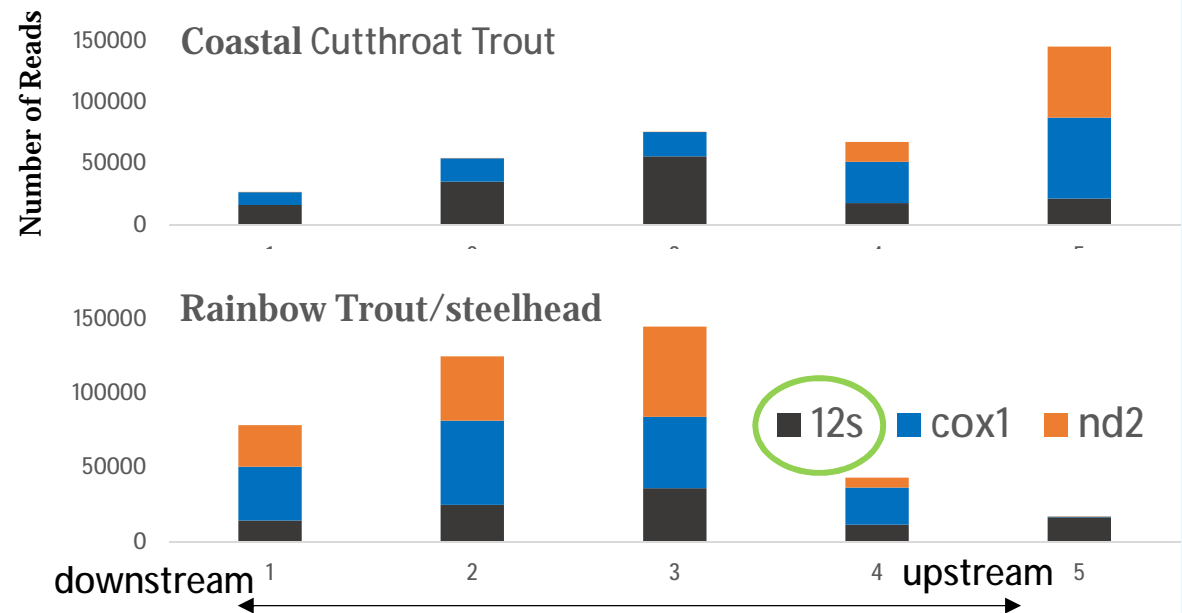
**Coho:** 3/240 bp mismatch = 1.25% divergence

# Salmonid Distributions Using Cytochrome Oxidase I (COI) using our Taxon-specific Primer

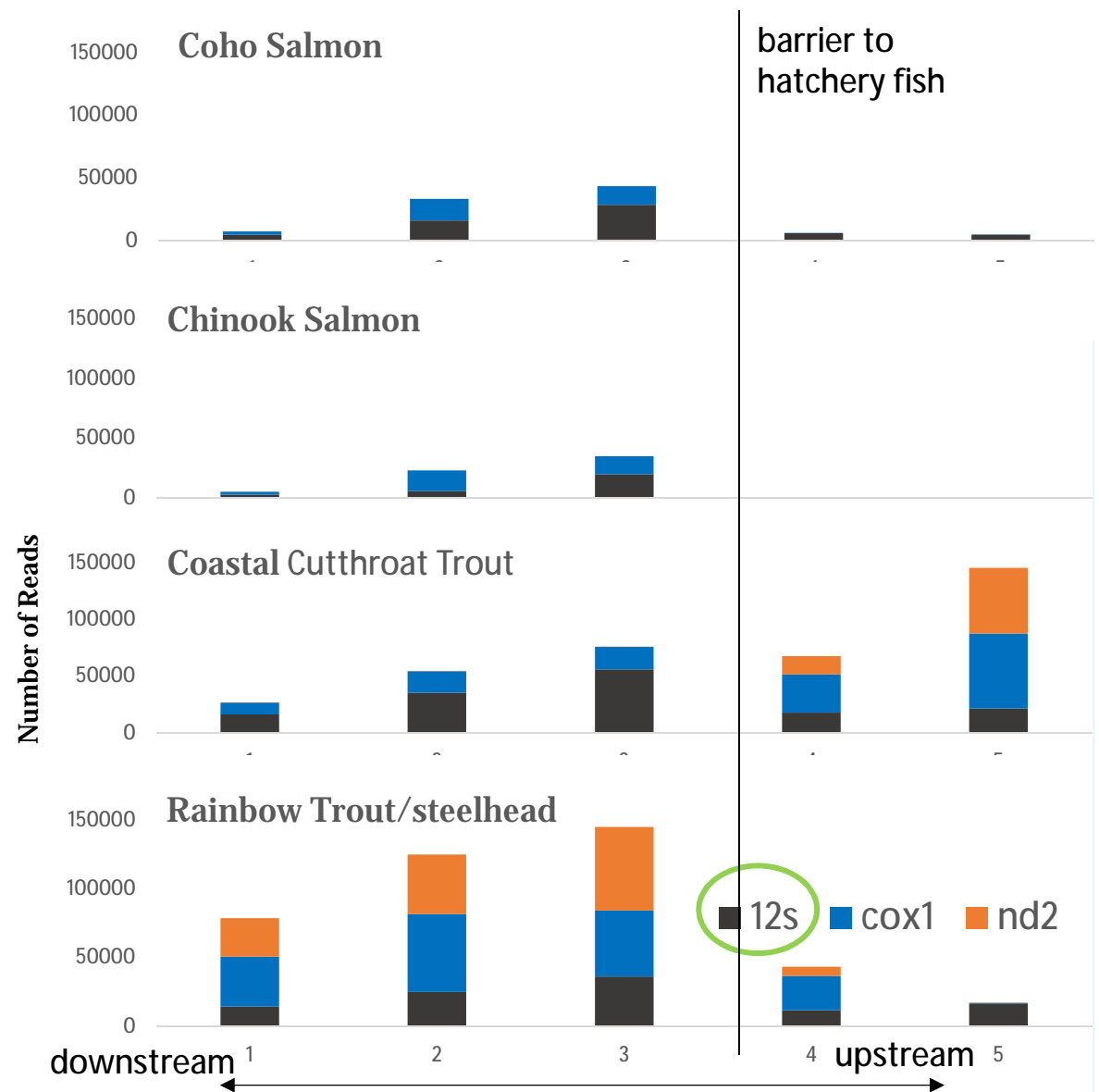


# eDNA detects changes in the fish assemblage along a stream with a barrier

Taxon-specific primers (cox1, nd2) are warranted to cross-validate taxon-general primers (12s) in eDNA metabarcoding approaches (Pacific trout)

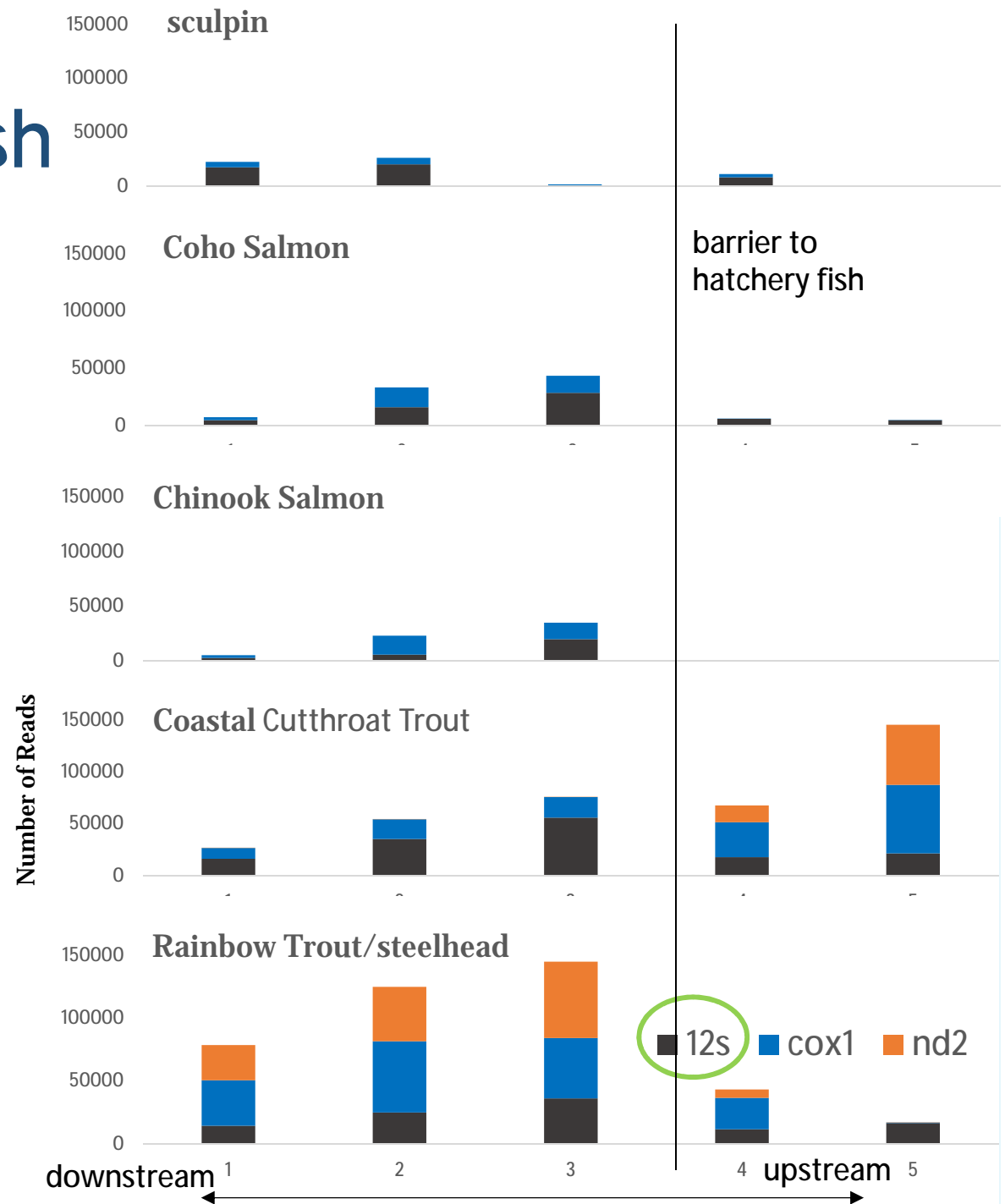


# eDNA detects changes in the fish assemblage along a stream with a barrier



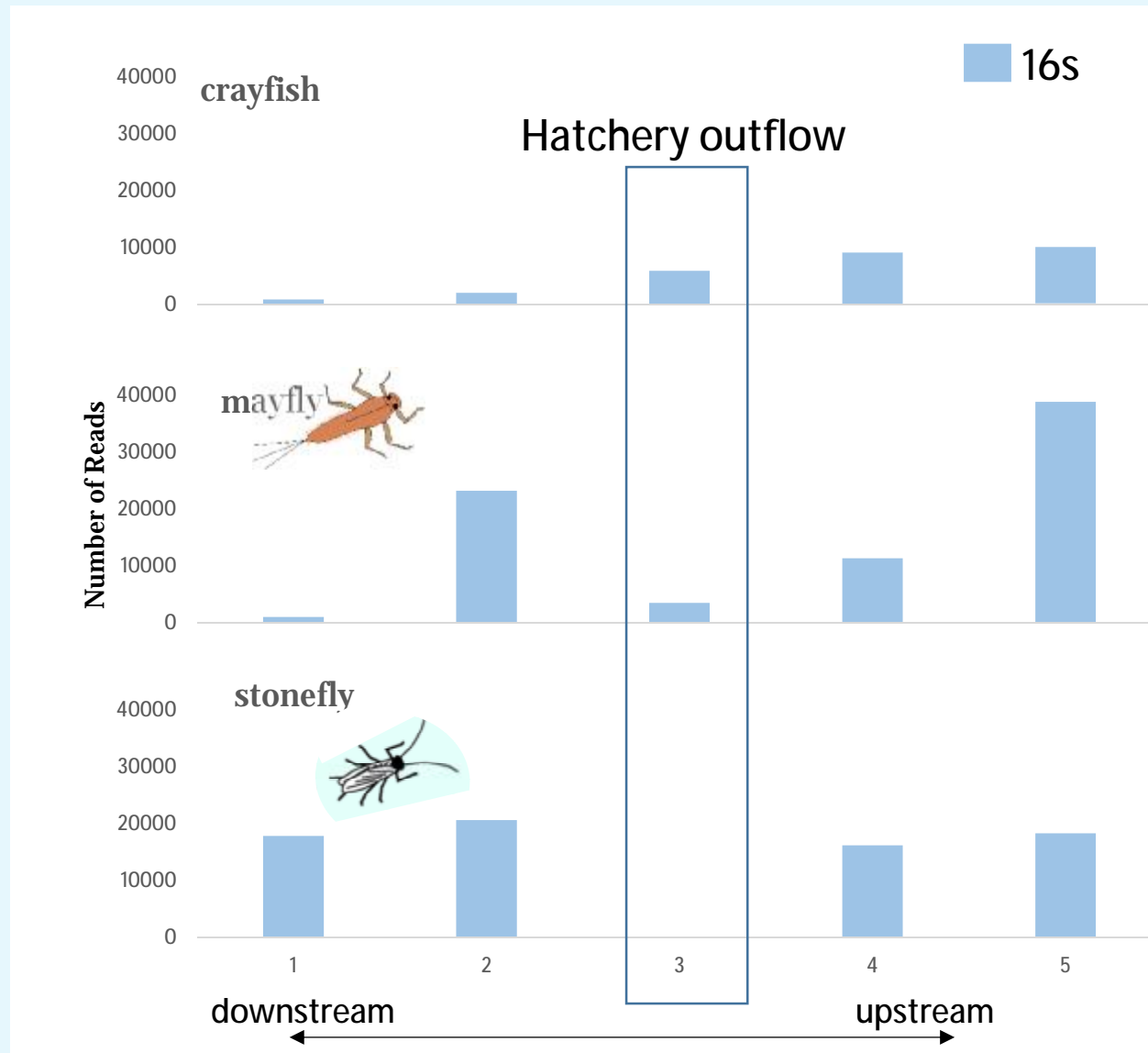
# eDNA detects changes in the fish assemblage along a stream with a barrier

Taxon-general primers are useful when genetic data is limited (sculpins)



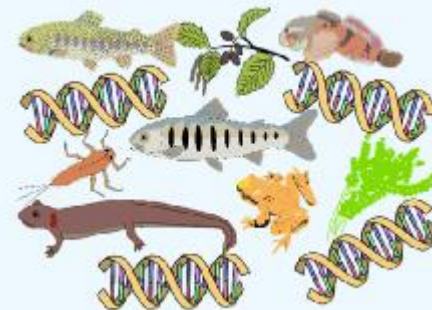


# eDNA detects changes in macroinvertebrates along a stream with a barrier



# Take home messages

- Preliminary results suggest utility for eDNA metabarcoding coupled with taxon-specific primers in identifying species from multiple taxon and tracking changes in aquatic biodiversity across a stream with a barrier
- Using multiple primer sets provides complementary views of species and a common ecosystem
- Multiple primer sets focusing on different subsets of taxa are necessary to sample an aquatic community in a reasonably comprehensive way



# Phase 2 of Proof-of-Concept Study: How well does our approach compare to traditional methods of electrofishing?

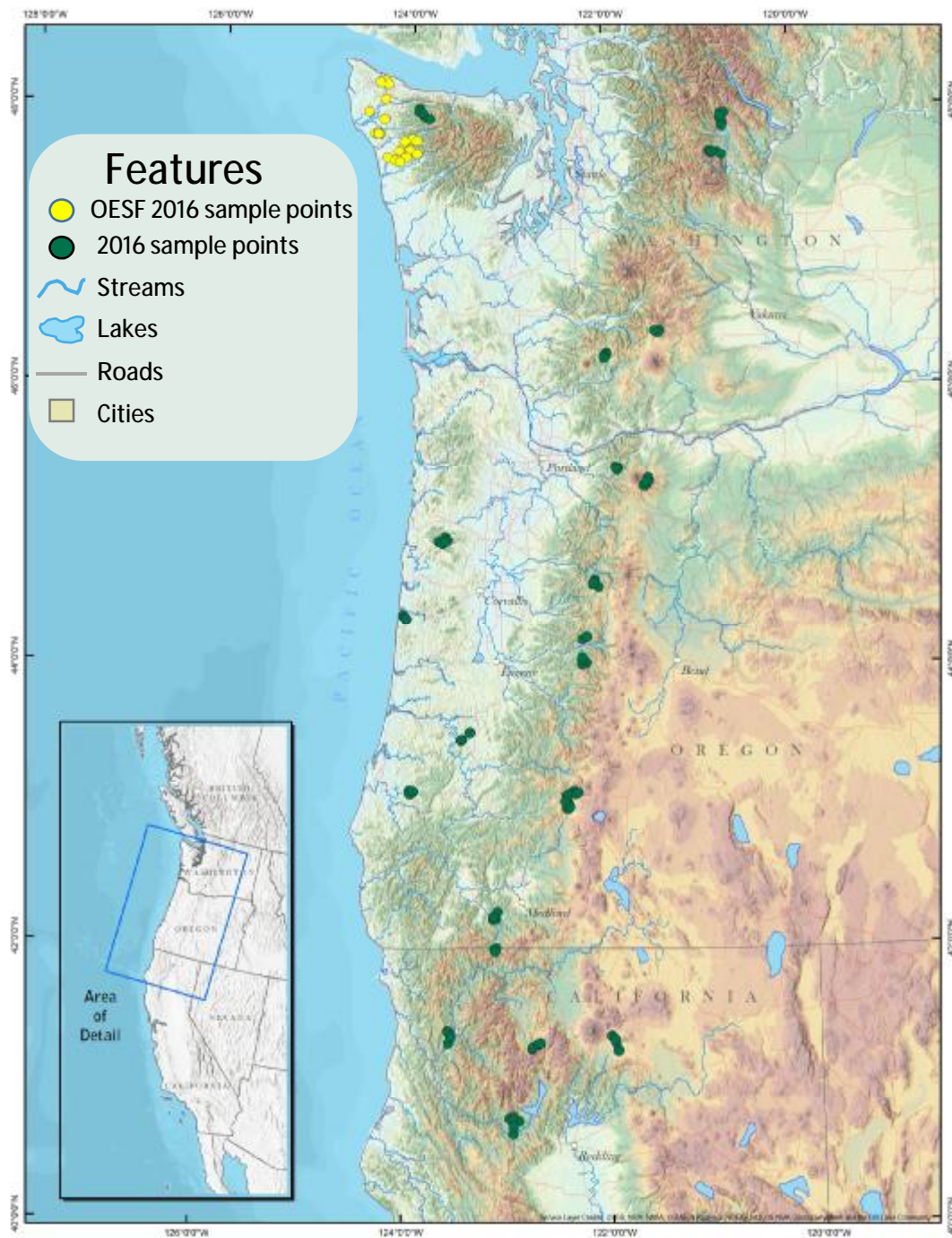
\*Tweak select primers and build pipeline for data management and analysis for all projects



vs

eDNA  
metabarcoding  
coupled with  
taxon-specific  
primers

Partnering with Oregon Department of Fish and Wildlife with Shaun Clements and Trevan Cornwell



Can we link occupancy of aquatic species in streams using eDNA to local habitat characteristics? Do downstream points integrate biodiversity data from upstream locations?

Collaborating with Tiffany Garcia (OSU)



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# Metabarcodes and taxon-specific primers

## n Fish

- **Metabarcoding:** Ray-finned teleosts 12S
- **Taxon specific:** *Oncorhynchus*, *Cottus*, *Acipenser*: with CO1, ND2, Cytb , D-Loop

## n Amphibians

- **Metabarcoding:** frog 12S and salamander 12S
- **Taxon specific:** *Ascaphus*, *Batrachoseps*, *Dicamptodon*, *Plethodon*, *Rana*, *Rhyacotriton*, *Taricha* with CO1, ND2, Cytb

## n Crayfish

- **Metabarcoding:** General Crayfish/Invertebrate 16S
- **Taxon specific:** *Pacifastacus*, *Procambarus*, and *Oronectes* CO1

## n Pathogens

- **Metabarcoding:** *Saprolegnia* 18S and *Myxobolus* 18S
- **Taxon specific:** *Phytophthora* (Cytb), *Phaeocryptopus* (tubulin), *Batrachochytrium* (ITS)