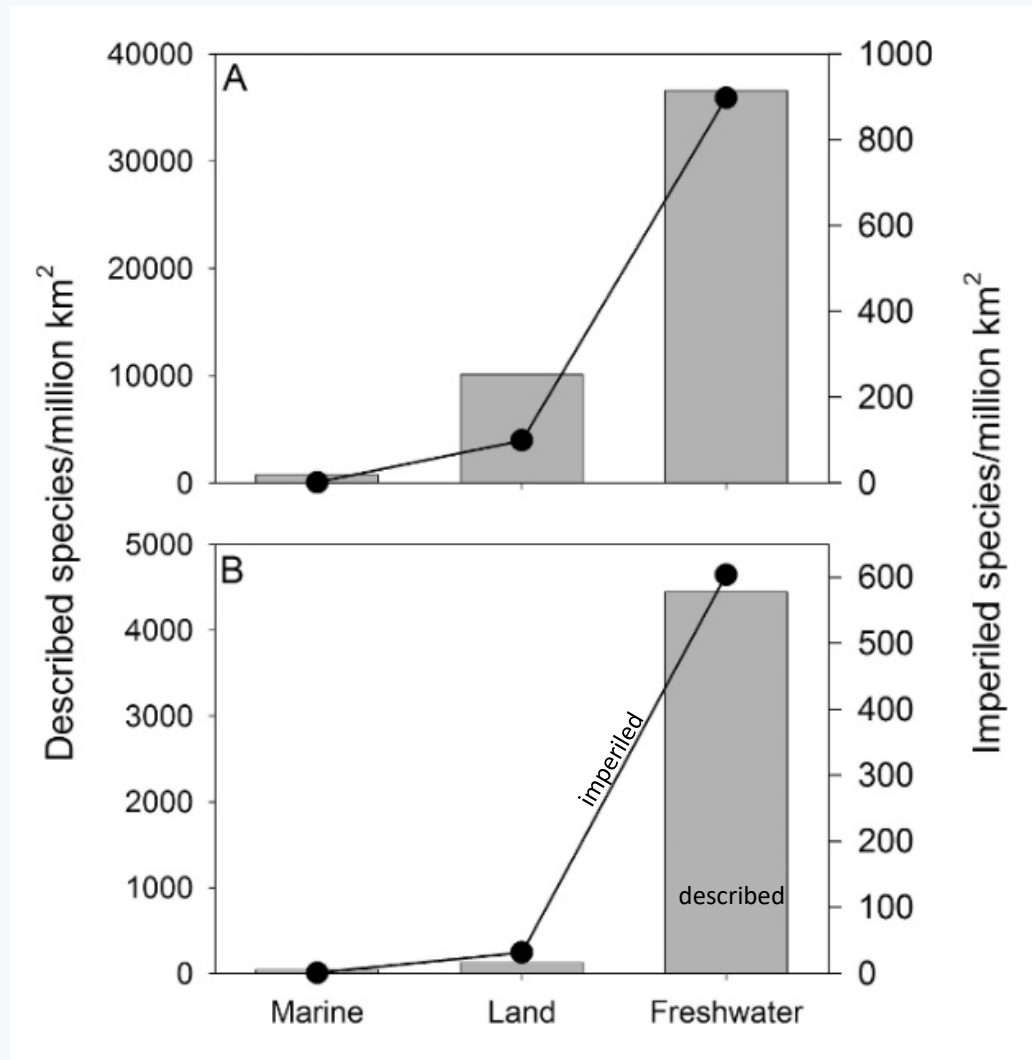


An Ecologists Guide to eDNA: Examples from Oregon

Brooke Penaluna
PNW Research Station
USDA Forest Service



Worldwide freshwater biodiversity is among most imperiled

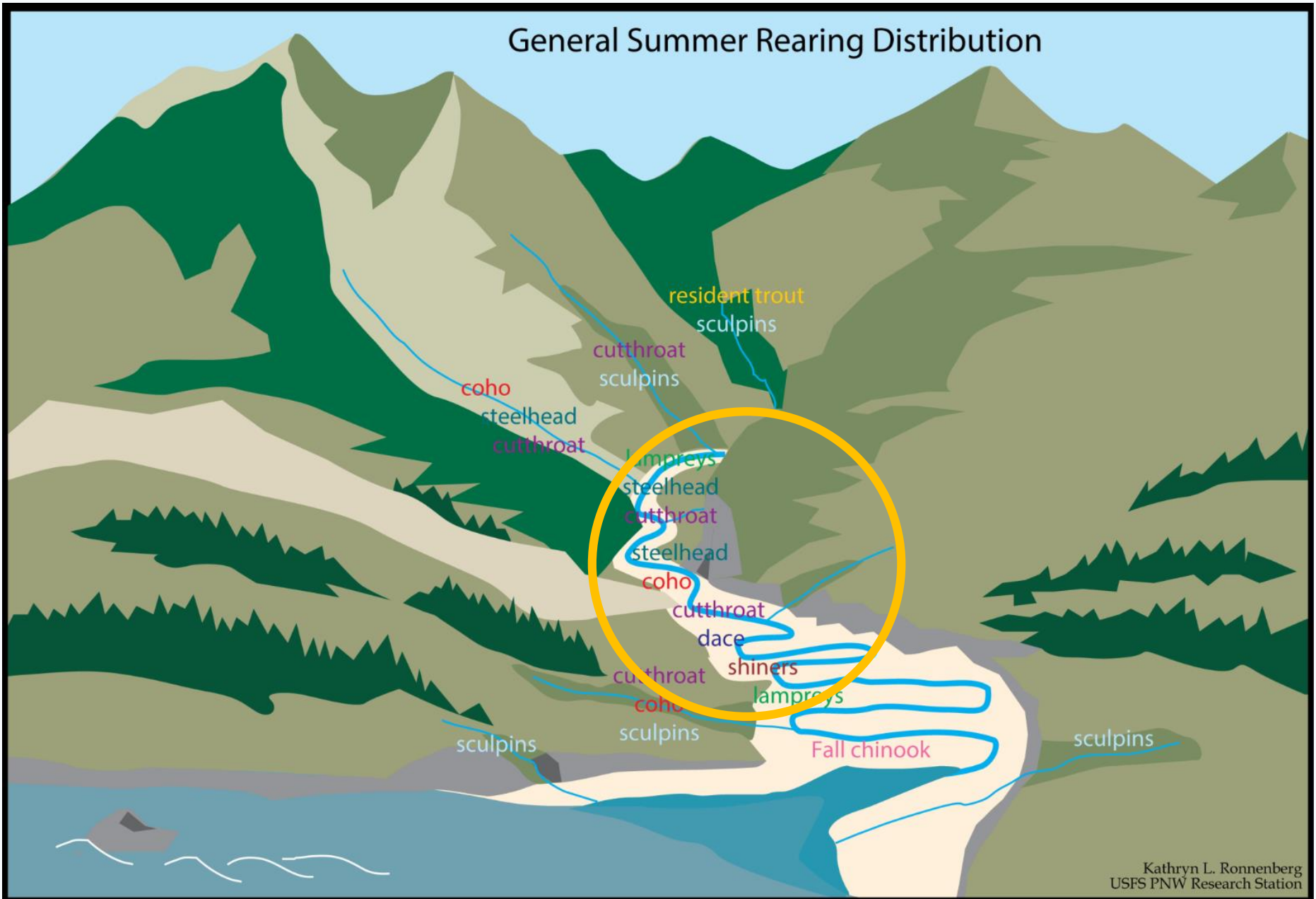


Traditional sampling for aquatic species

- 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



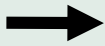
What is eDNA?

- Refers to DNA that can be extracted from soil, air, or water without isolating target organisms beforehand
- Water contains DNA of animals, plants, and microorganisms
- Think about: study system, focal species, and objectives of project

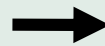


eDNA Barcoding: Single Species

Water Filtration



DNA Extraction

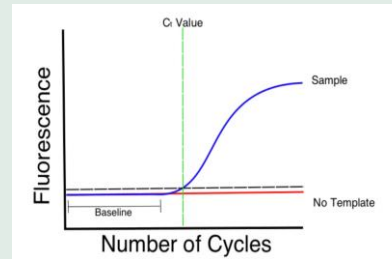


qPCR, ddPCR

Using primer for target species



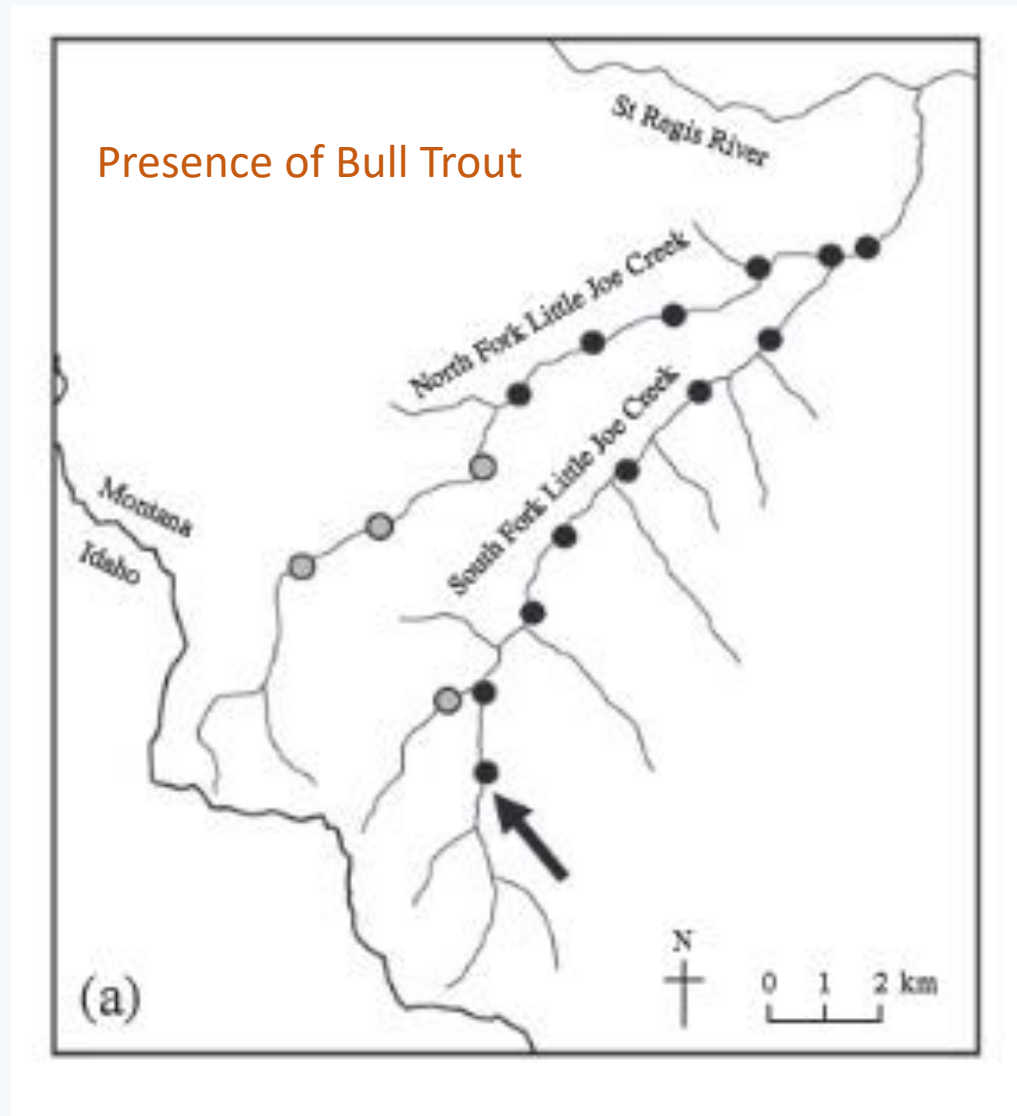
fluorescence of a single amplified gene that can be quantified



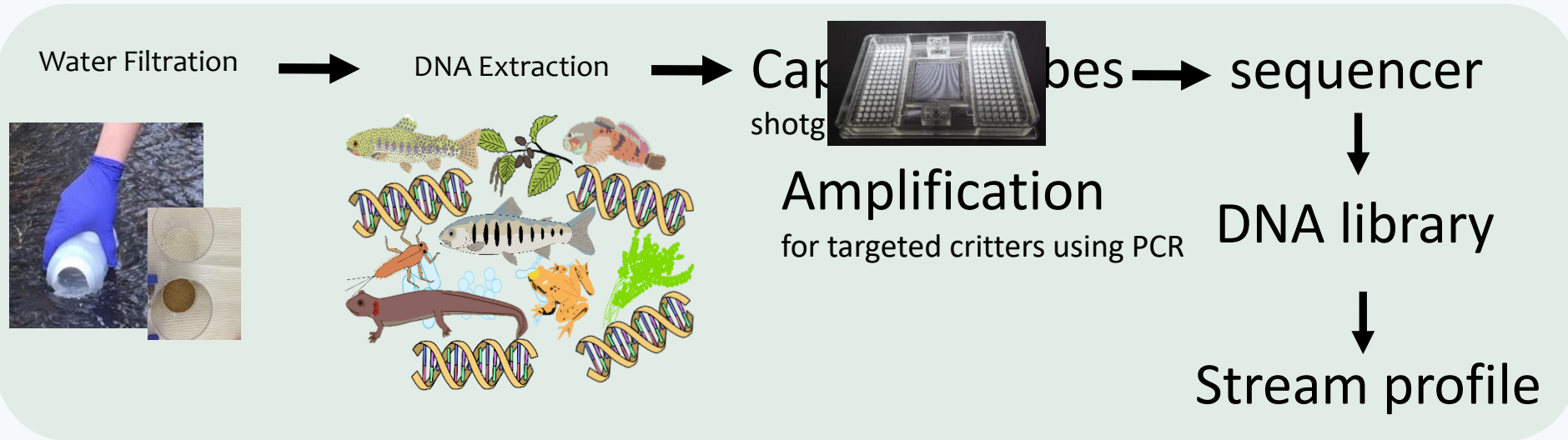
Established method: used in hundreds of published eDNA studies, diverse organisms

Cost: *inexpensive per species; expensive for multiple species*

eDNA is more sensitive than electrofishing



eDNA Metabarcoding: Multiple Species



Target: 100s - 1000s species per sample

Hybridization-capture of eDNA using 1000s of capture 'baits'

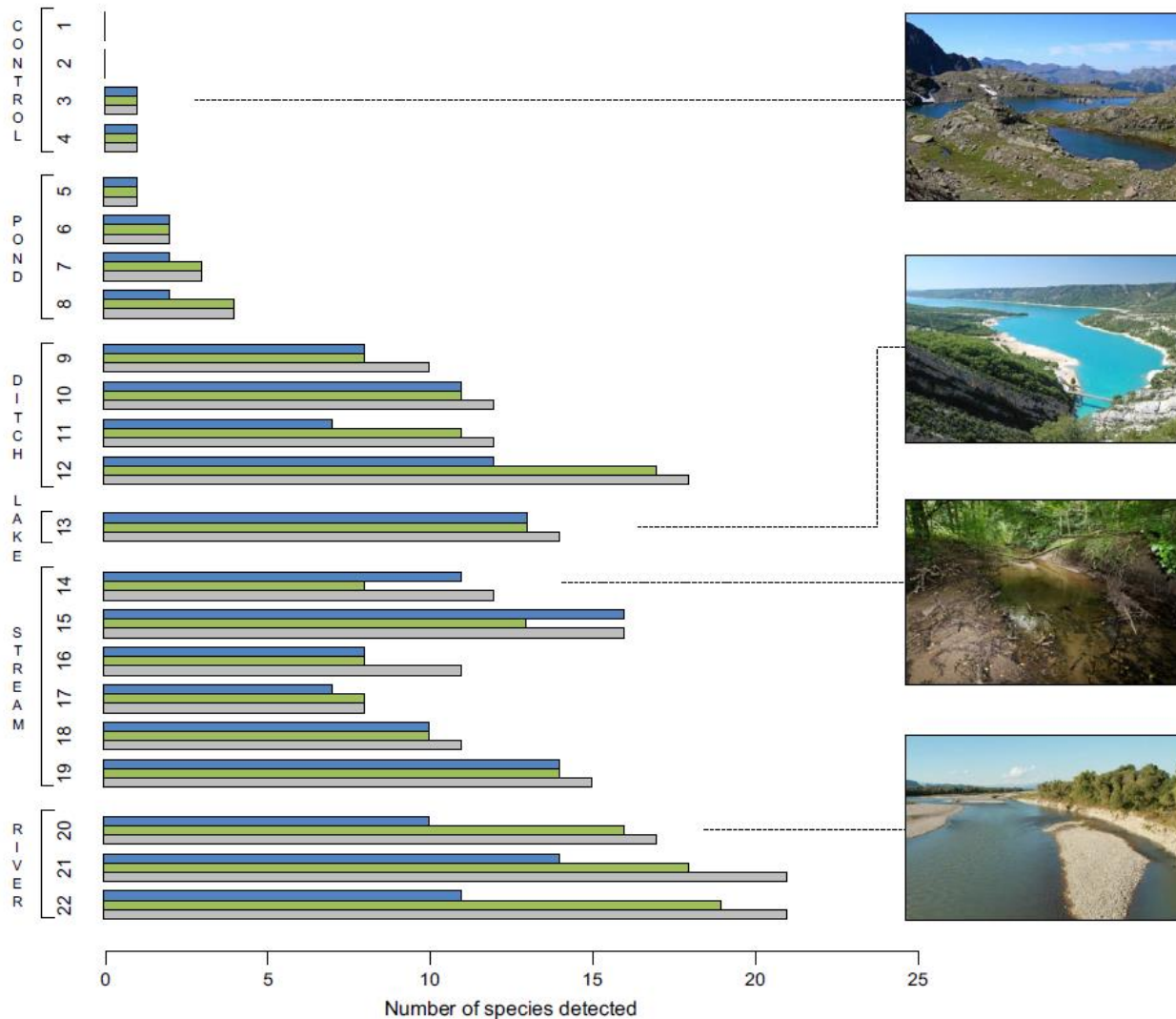
Detection: parallel DNA sequencing of hybridized products

Novel extension of hybridization: some published studies

Cost: *expensive per sample, but inexpensive per species*

Testing: USFS National Genomics Center

More fish species detected using eDNA metabarcoding than traditional surveys



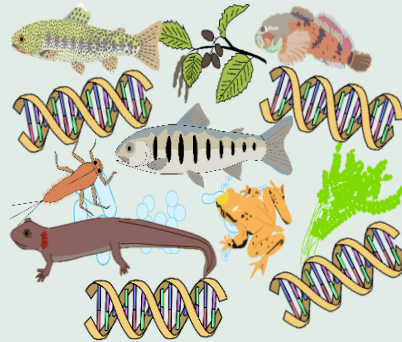
- Traditional surveys
- eDNA metabarcoding
Using 1 gene (12S)
- Total fishes from both approaches

eDNA Metabarcoding with both **universal** and **taxon-specific** primers

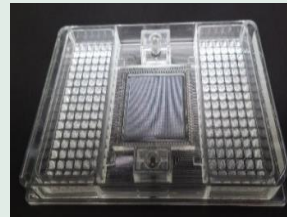
Water Filtration



DNA Extraction



DNA Amplification
of targets using PCR



48 targets x 48 samples
= 2,304 PCR reactions

DNA Sequencing
& Sequence Analysis

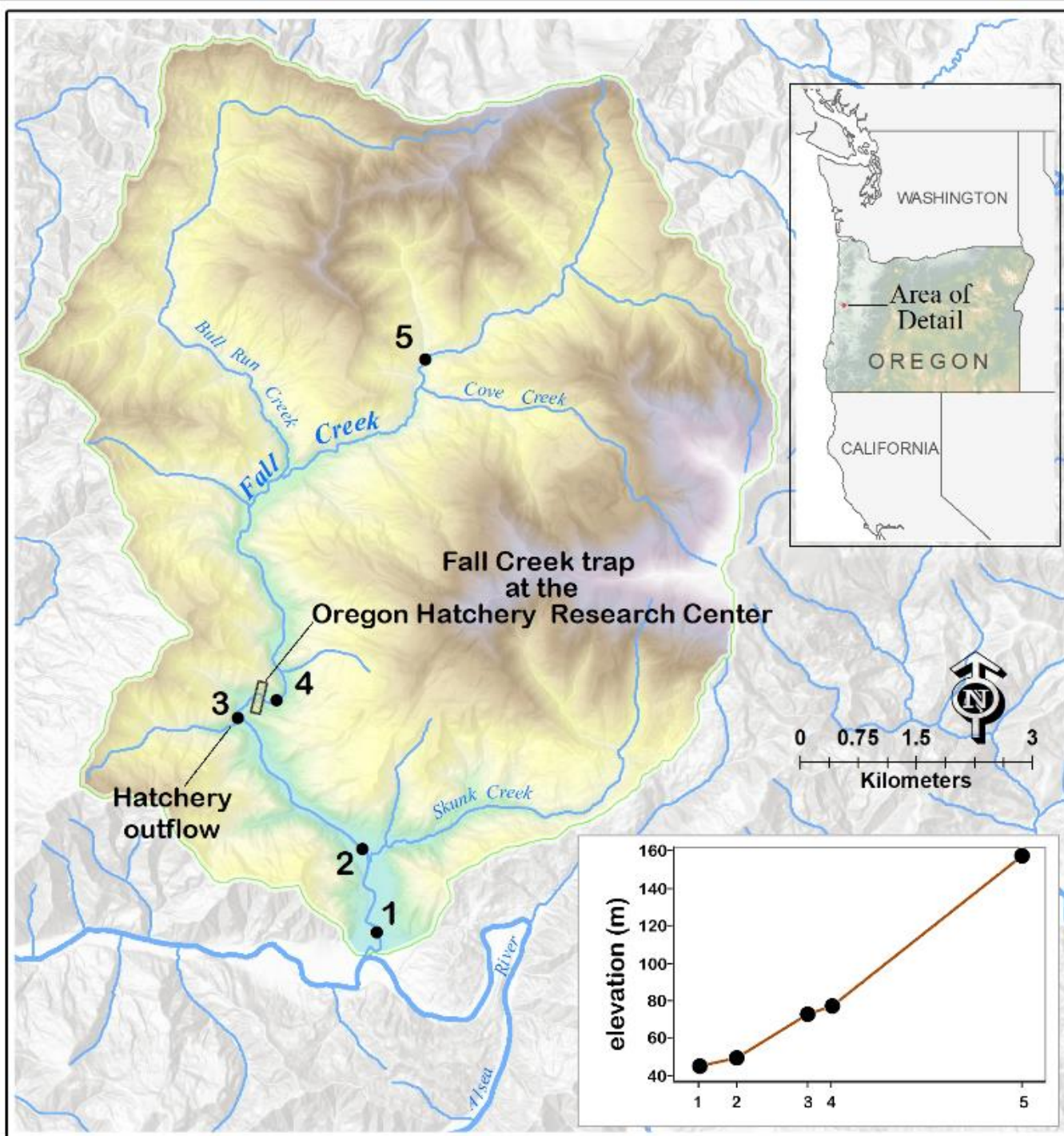


Stream Assemblage Profile

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

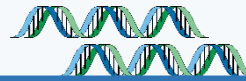
Compare electrofishing to multispecies eDNA





Weitmier et al.
under review

Electrofishing vs. eDNA



12S

COI

ND2

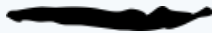
YOY

Cutthroat

Rainbow

Coho

Chinook



1 2 4 5

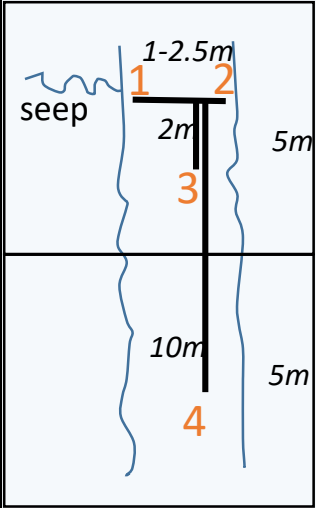
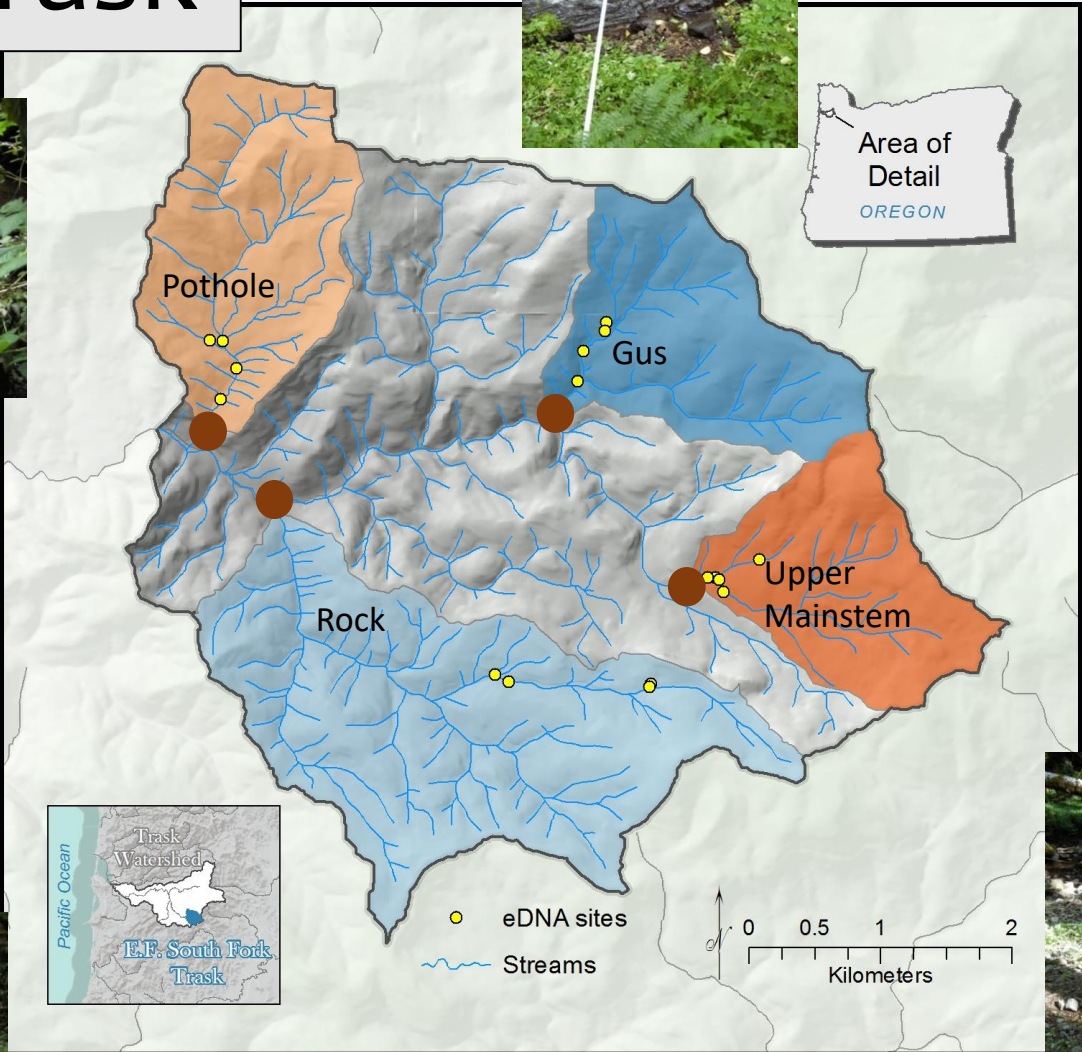
eDNA suggests Rainbow Trout are at upper elevation sites

Misidentification in field of sculpins, eDNA suggests more lineages of sculpins

Patchy detection of amphibians

Weitmier et al.
under review

Trask



Torrent Salamanders are generally detected in stream within first 10m of their seep

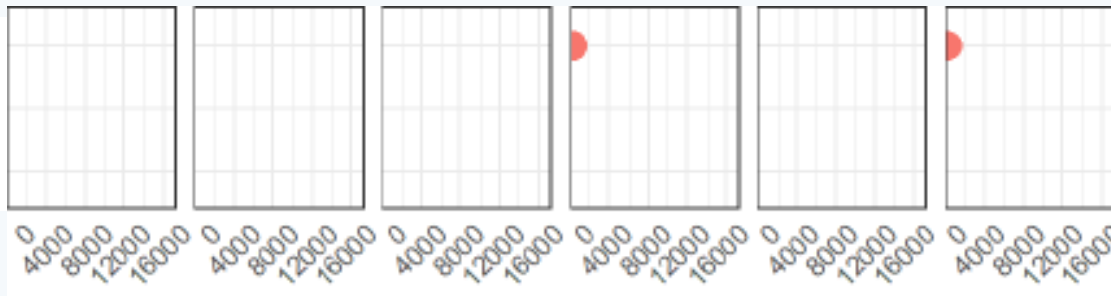
downstream

upstream

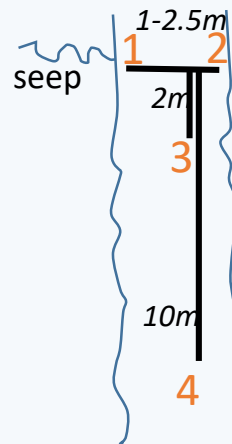
0m

865m

Torrent Salamander



Unknown -
Dicamptodon ND2 -
Ascaphus CytB -



Pothole



Taxon-general or universal



Taxon-specific


Take home messages

- Preliminary results suggest utility for multiple primer sets to detect species from multiple taxa
- **Built-in redundancy** when using multiple primers
- 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

Think about

- Study System (lake, river, reservoir, stream, pond)
- Focal Species (How does focal species use habitat?)
- Objectives of Project (Presence/Absence, relative abundance, Is there variability in ability to detect signal?)
- Pore size, volume of water to filter, replicates, multiple gene, sites



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Trask Watershed study and fellow Traskies

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