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

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US Forest Service
Acknowledgements

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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

- Resident trout
- Sculpins
- Coho
- Steelhead
- Cutthroat
- Lampreys
- Cutthroat
- Steelhead
- Coho
- Dace
- Shiners
- Lampreys
- Shiners
- Fall chinook

Kathryn L. Ronnenberg
USFS PNW Research Station
More fish species detected using eDNA metabarcoding

Valentini et al. 2016
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 → DNA Sequencing & Sequence Analysis

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

48 targets x 48 samples = 2,304 PCR reactions
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

Mapped Reads Per Species

- COI Coho
- COI Rainbow
- COI Cutthroat

barrier to hatchery fish

Above hatchery
Hatchery outflow
Below hatchery
Above confluence with Alsea River

Headwaters
Creek
Fall
Alsea River
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

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)
Metabarcodes and taxon-specific primers

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

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

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

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