Aquatic biodiversity from eDNA metabarcoding in response to Stage 0 restoration efforts at South Fork McKenzie River: A final grant report

Prepared by Brooke Penaluna, lead PI for eDNA monitoring and analyses

Part of the OWEB Grant # 220-7000-17342 of Evaluating Ecological and Geomorphic Responses to Stage 0 Restoration

Abstract

River restoration occurs throughout the globe to enhance habitats for native aquatic biodiversity and improve water quality. A specific type of restoration, named Stage 0 restoration, aims to reconnect floodplains to rivers by restoring fundamental processes that underly the river-floodplain corridor. However, most restoration projects occur without monitoring plans to understand successful aspects and lessons learned from those restoration actions, and consequently billions of dollars are spent without evaluating how the restoration activities have changed the river-floodplain aquatic communities. Stage 0 restoration is anticipated to create a diversity of aquatic habitat types that are likely to have distinct biological communities. Here, our goal was to identify the aquatic biodiversity of fishes, amphibians, mussels, crayfishes, and beaver from eDNA metabarcoding to understand the presence of aquatic species before and after restoration at South Fork McKenzie River (SFMR). We evaluated replicates at three transects during spring for four years to capture potential changes in the aquatic biodiversity richness following restoration compared to before. We identified a range of aquatic biodiversity with eDNA metabarcoding following restoration activities in two of the three transects at SFMR, including more detections of fishes, amphibians, mussels, crayfishes, and beaver suggesting that as a mosaic of habitats became available that a broader community of species occupied them. eDNA surveys detected 1 to 12 species per family across taxa. Although the eDNA metabarcoding results are descriptive and from a few restored reaches, they provide a line of evidence that habitat complexity begets biological diversity. As freshwater biomonitoring increasingly moves toward an ecosystem-based approach to understanding the effects of human impacts, such as restoration activities, eDNA data provides a more holistic survey than traditional approaches.

Introduction

Biodiversity has long been a proxy for measuring environmental change. Maintaining biodiversity in freshwaters is fundamental for safeguarding the productivity of many of the world's populations and ecosystems, including their resilience, and ability to adapt to environmental change. This is increasingly important as freshwater habitats have become degraded owing to human influences, which in some areas are now being actively restored. Restoration of aquatic habitats comes from large investments, especially for a type of restoration named Stage 0 that aims to reconnect floodplains to rivers. Unfortunately, most restoration projects occur without evaluating restoration actions, and consequently billions of dollars are spent without understanding how the restoration activities have changed the river-floodplain

aquatic communities. A global agenda for advancing freshwater biodiversity research has been presented indicating innovative methods for biodiversity assessments as a top priority (Maasri et al. 2022).

A cutting edge, innovative method includes environmental DNA (eDNA) metabarcoding, which is revolutionizing how we survey biodiversity by making species identification possible with high precision and accuracy. It is increasingly used to identify taxonomic lineages within broader taxonomic groups and to evaluate DNA diversity in a sample of water by identifying DNA that is left behind by organisms. eDNA metabarcoding of water uses the polymerase chain reaction (PCR) to amplify short, taxonomically informative genomic regions (DNA "barcodes") from eDNA. It is non-lethal and has been shown to detect more species than traditional approaches (Valentini et al. 2016). However, eDNA metabarcoding has not yet been used to evaluate freshwater biodiversity changes from restoration activities.

Here, we evaluate the freshwater biodiversity at three transects before and after restoration activities in the South Fork McKenzie River (SFMR) using eDNA metabarcoding described in Hauck et al. (2019) and Flitcroft et al. (in press). We targeted fish, amphibians, mussels, crayfishes, and beaver to understand how if and how those taxa change because of restoration activities. Ultimately, being able to better detect freshwater biodiversity allows managers the opportunity to recognize the diversity of species to ensure their persistence into the future.

Methods

The eDNA sampling at SFMR followed a transect-based approach to line up with surveys of substrate, elevation, and macroinvertebrate data. Three transects (1, 2, and 5) at SFMR were evaluated to understand aquatic biodiversity changes in response to restoration activities using eDNA surveys during pre- and post-restoration (Figure 1). We targeted fishes, amphibians, mussels, crayfishes, and beaver with eDNA metabarcoding. Although transects remained in the same geospatial position over time, the number and types of strata present on a transect changed in response to restoration (see strata details in macroinvertebrate report by Flitcroft et al. 2022). To ensure that sampling effort remained the same over time, duplicate eDNA samples were evaluated from each strata and pooled within their respective transect for each time period. Accordingly, the eDNA data was evaluated by summing species richness across strata for each transect during spring (April/May/June) from 2018 to 2021 (Tables 1 and 2).

To ensure that sampling effort remained the same over time, duplicate eDNA samples were evaluated from each strata and pooled within their respective transect for each time period. 500mL of water was pumped through 0.45 micron single–use cellulose nitrate filters (Sterlitech, Kent, WA, USA) using a vacuum pump. Filters were loosely rolled, stored frozen in 5mL vials on wet ice during collection and transport, and then frozen at –20°C within 6 hours of collection until DNA extraction. DNA was extracted from each filter using MoBio's PowerWater© DNA isolation kit (Qiagen, Hilden, Germany) per manufacturer's instructions. Post-extraction samples were cleaned and concentrated using the Zymoclean© Large Fragment DNA Recovery Kit (Zymo Research, Irvine, CA, USA).

We followed primer design as in Weitemier et al. (2021). We used dbcAmplicons version 0.9.1 (Settles & Gerritsen, 2014, https://github.com/msettles/dbcAmplicons) to sort reads from each sample by primer, trim primer and adapter sequences, trim 3' regions with Phred quality

<20, and remove reads <50 bp. To reduce the influence of sequencing error and barcode swaps, we only retained reads that perfectly matched a sample barcode. We allowed up to 6 bp differences (Hamming distance) between expected and sequenced primers. We overlapped read pairs using the dbcAmplicons join function, allowing ≤25% mismatches in the overlapped region. We used bbduk2 version 38.58 (Bushnell and Rood, 2019) to further filter overlapped reads by removing any PhiX reads, trimming any remaining adapters or barcodes, and removing reads >27 bp shorter than expected for their primer. Reads from "universal" primers targeting ribosomal or spacer regions were removed if they were <99 bp. Following read processing, reads were classified to taxon of origin using the program KMA version 1.3.9 (Clausen et al, 2018, https://doi.org/10.1186/s12859-018-2336-6) and a database containing all ncbi nt entries from January 2018, excluding entries from "environmental eukaryotes, environmental prokaryotes, unclassified sequences, and artificial sequences" (Marcelino et al, 2019, https://doi.org/10.25910/5cc7cd40fca8e). We used CCMetagen version 1.2.5 (Marcelino et al., 2019, https://doi.org/10.1186/s13059-020-02014-2) to summarize classification results create a ranked taxonomy.

We applied a read threshold of <25 from the negative control samples allowing us to account for some errors (e.g., lab or sample contamination; sample barcode swaps). The read threshold does not account for database uncertainty for taxa that are only differentiated by a few SNPs, or that share haplotypes across divergent species. We extracted the fish, amphibian, mussel, crayfish, and beaver data for Transects 1, 2, and 5 from springtime sampling from the raw data. We filtered the extracted database to only include detections for taxa found in the Pacific Northwest of North America. We described aquatic biodiversity as the sum of the species richness information across strata for each transect during spring of each year sampled. White Sturgeon was assumed to be the species detected in the Acipenseridae family as it is the only potential freshwater sturgeon upstream in the Willamette River watershed.

Results

Aquatic biodiversity changed in species richness across taxa post-restoration relative to pre-restoration at SFMR across fishes, amphibians, mussels, crayfishes, and beaver (Figure 2; Tables 1 and 2). Species richness was orders of magnitude higher post-restoration in Transects 2 and 5, while for Transect 1 pre-restoration richness was higher. There were more amphibians, crayfishes, and mussels post-restoration at Transects 2 and 5, whereas these taxa went undetected pre-restoration. Transects 1 and 5 showed an increase in number of species post-restoration over time, whereas Transect 2 had relatively equal species detections across years post-restoration. Overall, eDNA surveys detected 1 to 12 species per family across taxa.

Discussion

A compelling range of aquatic biodiversity was detected with eDNA metabarcoding following restoration activities in two of three transects at SFMR, including more detections of fishes, amphibians, mussels, crayfishes, and beaver suggesting that as a mosaic of habitats became available a broader community of species occupied them. Transect 1 had higher diversity pre-restoration likely owing to its location above the confluence with the mainstem McKenzie River, as areas around river confluences have more diversity (Kiffney et al. 2006). As time passes post-restoration, it is possible that species detections will continue to increase at Transect

1 potentially matching pre-restoration numbers as it is possible that that area is still recovering. Of the species detected, rare, common, threatened, and invasive species were all identified. As an example, invasive American Bullfrog were detected at Transect 5 post-restoration as they likely moved upstream from Transect 1 as habitats were created and became available. Although the eDNA metabarcoding results are descriptive and from a few restored reaches, they provide a line of evidence that habitat complexity begets biological diversity. Repopulation by beaver was detected at Transect 5 with eDNA results suggesting that restoration of processes needed to sustain them are found there following restoration activities.

Plans for continued monitoring

There are no plans for continued eDNA monitoring. We lack the required resources to maintain continued sampling without funds to complete the work.

Lessons learned

The eDNA sampling approach would have been enhanced by consistent collection across habitats and time periods. The data we currently have is sufficient to make a comparison in spring across years for three transects, but consistent sampling across seasons and years are important to understand how a community of fishes uses restored habitats seasonally. For example, what taxon use the habitats in fall or winter and which season do species recolonize the habitats following restoration? Also, it would be important to understand more about the pool of potential aquatic species that could use the restored habitats by sampling downriver of the restored reaches. In the case of SFMR, we could have sampled at the confluence of the McKenzie River or in the upper Willamette River.

Recommendations

We strongly recommend that researchers and managers work together to develop monitoring plans for restoration activities, including multiple years of pre-restoration data to more completely evaluate the change that restoration creates. Future research can evaluate whether enhanced aquatic biodiversity is more generally found across multiple restored sites using eDNA metabarcoding.

Conclusions

This study enhances the biogeography understanding of aquatic biodiversity following Stage 0 restoration. We showed that aquatic biodiversity can be comprehensively inventoried using eDNA metabarcoding, allowing for multiple species to be assess simultaneously. Our work supports the idea that a diversity of habitats resulting from Stage 0 restoration supports a broader biological community of species.

Acknowledgments

Rich Cronn provided insights at all phases of eDNA development, Laura Hauck developed eDNA metabarcoding primers, Kevin Weitemier developed the eDNA data pipeline, and Lucas Longway completed extractions. We thank Dr Mark Band and the University of Illinois at

Urbana-Champaign Roy J. Carver Biotechnology Center for DNA amplification, sequencing, and consultation. Computational infrastructure was supported and maintained by the Oregon State University Center for Genome Research and Biocomputing. Jared Weybright and the McKenzie Watershed Council for coordinating and administering the funding related to the OWEB grant.

References

Hauck, L. L., Weitemier, K. A., Penaluna, B. E., Garcia, T. S., and Cronn, R. (2019). Casting a Broader Net: Using Microfluidic Metagenomics to Capture Aquatic Biodiversity Data from Diverse Taxonomic Targets. *Environmental DNA 1, 251-267.*

Flitcroft, R., W. R. Brignon, B. Staab, R. Bellmore, J. Burnett, P. Burns, B. Cluer, G. Giannico, M. Helstab, J. Jennings, C. Mayes, C. Mazzacano, L. Mork, K. Meyer, J. Munyon, B. Penaluna, P. Powers, D. N. Scott, and S. Wondzell. (2022). Rehabilitating valley floors to a stage 0 condition: A synthesis of opening outcomes. Frontiers in Environmental Science.

Kiffney, P. M., Greene, C. M., Hall, J. E., & Davies, J. R. (2006). Tributary streams create spatial discontinuities in habitat, biological productivity, and diversity in mainstem rivers. Canadian Journal of Fisheries and Aquatic Sciences, 63(11), 2518-2530.

Kim, D., Song, L., Breitwieser, F. P., and Salzberg, S. L. (2016). Centrifuge: Rapid and Sensitive Classification of Metagenomic Sequences. *Genome Research 26, 1721-1729.*

Maasri, A., Jähnig, S. C., Adamescu, M. C., Adrian, R., Baigun, C., Baird, D. J., ... & Worischka, S. (2022). A global agenda for advancing freshwater biodiversity research. Ecology letters, 25(2), 255-263.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., ... & Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Molecular ecology, 25(4), 929-942.

Weitemier K., Penaluna B.E., Hauck L.L., Longway L.J., Garcia T., Cronn R. (2021). Estimating the genetic diversity of Pacific salmon and trout using multigene eDNA metabarcoding. Molecular Ecology 30:4970-4990.

Outreach

Presentations, workshops, or fields trips

Penaluna, B.E. 2022. The Promise of eDNA. Results from the eDNA metabarcoding at SFMR are featured in this presentation that covers a collection of eDNA results across the Pacific Northwest. PNW SciCast, a webinar series of the Pacific Norwest Research Station. June 30, 2022.

Published papers and/or reports

Flitcroft, R., W. R. Brignon, B. Staab, R. Bellmore, J. Burnett, P. Burns, B. Cluer, G. Giannico, M. Helstab, J. Jennings, C. Mayes, C. Mazzacano, L. Mork, K. Meyer, J. Munyon, B. Penaluna,

P. Powers, D. N. Scott, and S. Wondzell. In press. Rehabilitating valley floors to a stage 0 condition: A synthesis of opening outcomes. Frontiers in Environmental Science.

Funding

Cash contribution

$32,000 from Pacific Northwest Station, USDA Forest Service in a Joint Venture Agreement to Oregon State University

$40,000 from OWEB to McKenzie Watershed Council to support the eDNA work.

In-kind contribution

Labor Project oversight, field sampling, primer development, data management, and data analyses came from various USFS personnel over the duration of the eDNA monitoring part of the project.

Materials Supplies and materials were purchased as needed for this project, including extraction kits and lab supplies, and running the samples on the machines at the University of Illinois at Urbana-Champaign Roy J. Carver Biotechnology Center for DNA amplification, sequencing, and consultation.

Figure 1. Map of eDNA sampling sites at Southfork McKenzie River. eDNA was collected in strata at transects 1, 2, and 5, which also coincides with macroinvertebrate sampling sites.

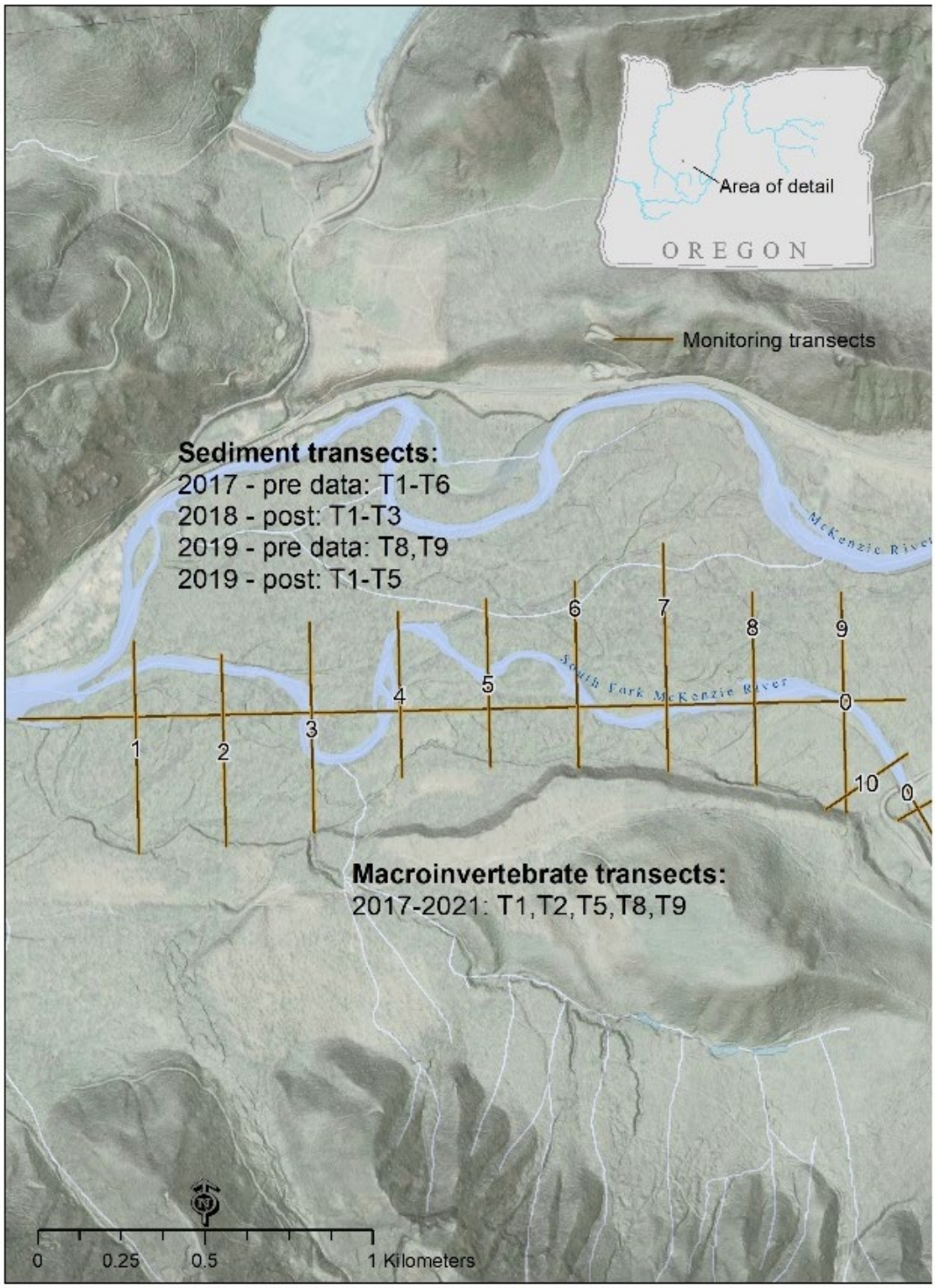
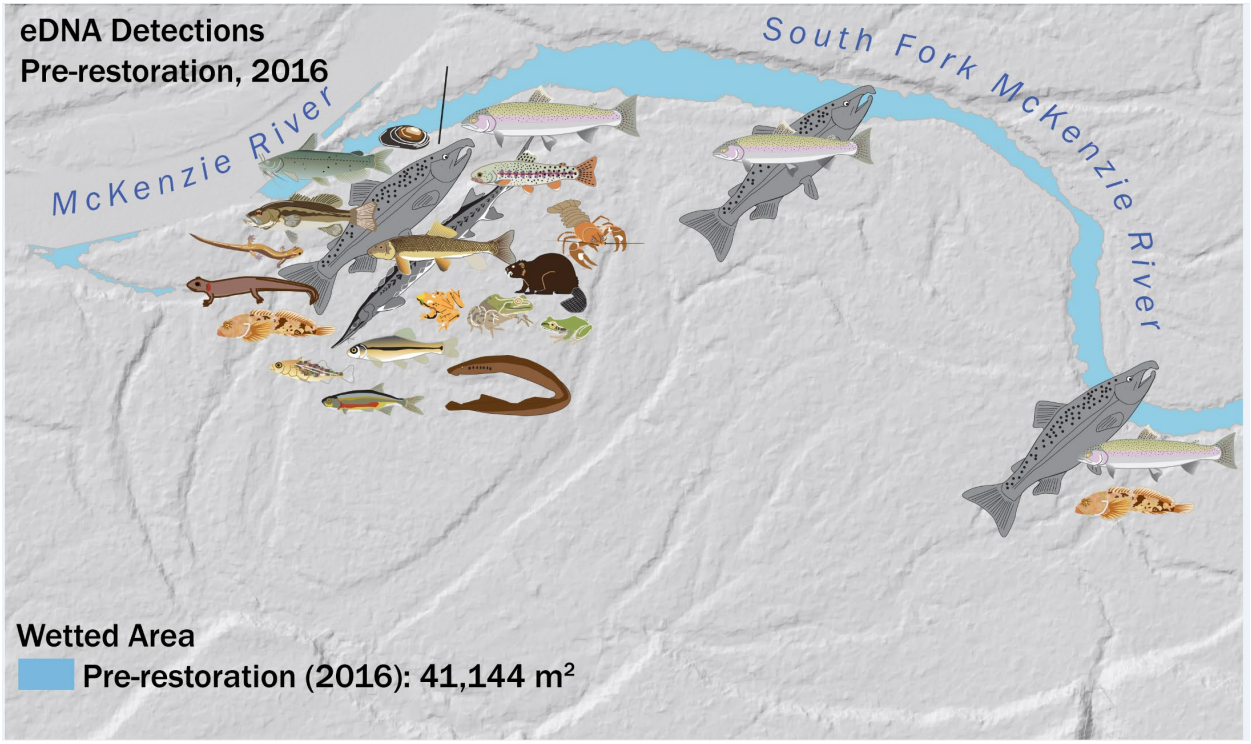


Figure 2. Visualization of eDNA detections pre- and post-restoration at South Fork McKenzie River for transects 1,2, and 5.



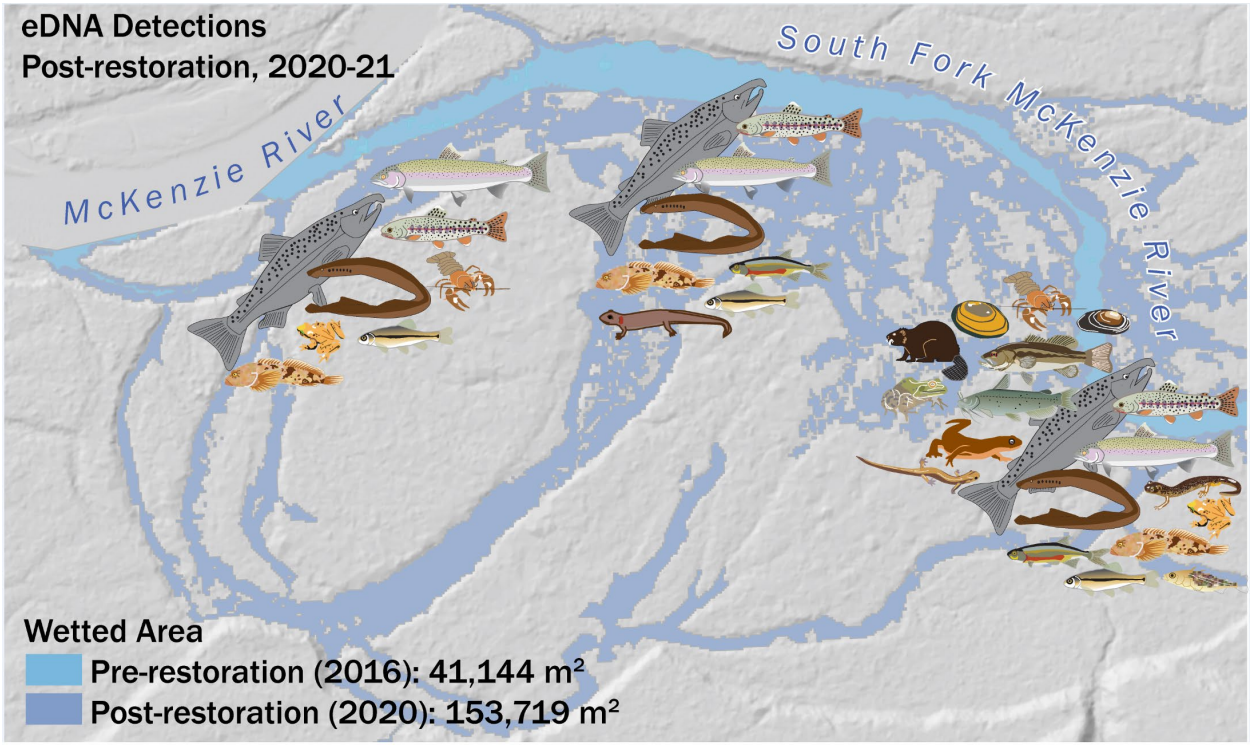


Table 1. Aquatic Biodiversity richness from eDNA metabarcoding of species detections grouped within their taxonomic family during spring at three transects that underwent restoration activities at the South Fork McKenzie River. Post-restoration started spring of 2019 at Transects 1 and 2 and in spring of 2020 at Transect 5 (grayed cells). Duplicate samples were analyzed from each strata along a transect for each time period and pooled within their respective transect/time period.

Taxonomic Family	Total # of species	Transect 1				Transect 2				Transect 5			
		Pre-restoration	Post-restoration			Pre-restoration	Post-restoration			Pre-restoration		Post-restoration	
		2018	2019	2020	2021	2018	2019	2020	2021	2018	2019	2020	2021
Salmonidae (Salmonids)*	8	7	4	4	4	1	2	4	3	3	1	4	7
Acipenseridae (Sturgeons)	1	1											
Catostomidae (Suckers)	1	1											
Petromyzontidae (Northern Lampreys)	3	3	2		2		2	2	2				2
Cottidae (Sculpins)*	12	7	1	4	5		3	2	3	1		3	8
Leuciscidae (True Minnows)*	3	1	1		2		1	1				1	1
Gasterosteidae (Stickelbacks)	1	1											1
Umbridae (Pike and Mudminnows)*	1	1											1
Cyprinidae (Carps and minnows)	1	1	1		1			1	1			1	1
Ictaluridae (Catfishes)	1	1											1
Centrachidae (Sunfishes)	3	3											1
Ascaphidae (Tailed Frogs)	1	1			1								1
Ranidae (True Frogs)	3	3											3
Hylidae (Tree Frogs and allies)	1	1					1						
Ambystomatidae (Salamanders)	1	1											
Salamandridae (True Salamanders)	1	1											2
Rhyacotritonidae (Torrents)*	4	2											3
Plethodontidae (Lungless Salamanders)	2	2											2
Astadidae (Freshwater Crayfishes)	1	1	1	1	1								1
Cambaridae (Freshwater Crayfishes)	4	1										1	1

Unionidae (Mollusks)*	2	1											2
Margaritiferidae (Freshwater Mussels)	1	1											1
Castoridae (Beavers)	1												1
Total	**57**	**41**	**10**	**9**	**16**	**1**	**9**	**10**	**9**	**4**	**1**	**10**	**39**
# of replicates		8	12	12	12	2	8	8	8	2	10	10	10

*likely taxonomic and/or misclassification issues within family

Table 2. Source data of eDNA detections of species at the South Fork McKenzie River. Post-restoration started in 2019 at Transects 1 and 2 and in 2020 at Transect 5. Duplicate samples were analyzed from each strata along a transect for each time period and pooled within their respective transect/time period. *Likely taxonomic or misclassification issues.

Scientific Name	Common Name	Family	Transect 1				Transect 2				Transect 5			
			2018	2019	2020	2021	2018	2019	2020	2021	2018	2019	2020	2021
Oncorhynchus clarkii	Coastal Cutthroat Trout	Salmonidae	1	1	1	1	0	0	0	1	0	0	1	1
Oncorhynchus kisutch	Coho Salmon	Salmonidae	1	0	1	0	0	0	1	0	1	0	0	0
Oncorhynchus mykiss	Rainbow Trout/steelhead	Salmonidae	1	1	1	1	1	1	1	1	1	0	1	1
Oncorhynchus tshawytscha	Chinook Salmon	Salmonidae	1	1	1	1	0	1	1	1	1	1	1	1
Prosopium williamsoni	Mountain Whitefish	Salmonidae	1	1	0	1	0	0	1	0	0	0	1	1
Salmo trutta	Brown Trout	Salmonidae	0	0	0	0	0	0	0	0	0	0	0	1
*Salvelinus fontinalis**	Brook Trout	Salmonidae	1	0	0	0	0	0	0	0	0	0	0	1
*Salvelinus malma**	Dolly Varden	Salmonidae	1	0	0	0	0	0	0	0	0	0	0	1
Acipenser	Sturgeon genus	Acipenseridae	1	0	0	0	0	0	0	0	0	0	0	0
Catostomus macrocheilus	Largescale Sucker	Catostomidae	1	0	0	0	0	0	0	0	0	0	0	0
Lampetra ayresii	River Lamprey	Petromyzontidae	1	1	0	1	0	1	1	1	0	0	0	1
Lampetra richardsoni	Western Brook Lamprey	Petromyzontidae	1	0	0	0	0	0	1	0	0	0	0	0
Entosphenus tridentatus	Pacific Lamprey	Petromyzontidae	1	1	0	1	0	1	0	1	0	0	0	1
*Cottus aleuticus**	Coastrange Sculpin	Cottidae	1	0	0	1	0	0	0	0	0	0	0	1
*Cottus asper**	Prickly Sculpin	Cottidae	1	0	1	1	0	0	0	0	1	0	1	1
*Cottus bairdii**	Mottled Sculpin	Cottidae	1	1	1	1	0	1	0	1	0	0	1	1
*Cottus bendirei**	Malheur Sculpin	Cottidae	0	0	0	0	0	0	0	0	0	0	0	1
*Cottus confusus**	Shorthead Sculpin	Cottidae	0	0	0	1	0	0	1	1	0	0	1	0
*Cottus cognatus**	Slimy Sculpin	Cottidae	0	0	1	0	0	0	0	0	0	0	0	1
*Cottus gulosus**	Riffle Sculpin	Cottidae	1	0	0	1	0	0	1	0	0	0	0	1
*Cottus hubbsi**	Columbia Sculpin	Cottidae	0	0	1	0	0	1	0	1	0	0	0	1
*Cottus marginatus**	Margined Sculpin	Cottidae	1	0	0	0	0	0	0	0	0	0	0	0
*Cottus perplexus**	Reticulate Sculpin	Cottidae	1	0	0	0	0	0	0	0	0	0	0	0
*Cottus pitensis**	Pit Sculpin	Cottidae	1	0	0	0	0	0	0	0	0	0	0	0

*Cottus rhotheus**	Torrent Sculpin	Cottidae	0	0	0	0	0	1	0	0	0	0	0	1
Rhinichthys cataractae	Longnose Dace	Leudiscidae	0	0	0	1	0	0	0	0	0	0	0	0
Rhinichthys osculus	Speckled Dace	Leudiscidae	0	1	0	1	0	1	1	0	0	0	1	1
Richardsonius balteatus	Redside Shiner	Leudiscidae	1	0	0	0	0	0	0	0	0	0	0	0
Pimephales promelas	Fathead Minnow	Cyprinidae	1	1	0	1	0	0	1	1	0	0	1	1
Gasterosteus aculeatus	Threespine stickelback	Gasterosteidae	1	0	0	0	0	0	0	0	0	0	0	1
Ictalurus punctatus	Channel Catfish	Ictaluridae	1	0	0	0	0	0	0	0	0	0	0	1
Micropterus salmoides	Largemouth Bass	Centrachidae	1	0	0	0	0	0	0	0	0	0	0	1
Lepomis macrochirus	Bluegill	Centrachidae	1	0	0	0	0	0	0	0	0	0	0	0
Perca flavescens	Yellow Perch	Centrachidae	1	0	0	0	0	0	0	0	0	0	0	0
*Novumbra hubbsi**	Olympic Mudminnow	Umbridae	1	0	0	0	0	0	0	0	0	0	0	1
Ascaphus truei	Coastal Tailed Frog	Ascaphidae	1	0	0	1	0	0	0	0	0	0	0	1
Taricha granulosa	Rough-skinned Newt	Salamandridae	0	0	0	0	0	0	0	0	0	0	0	1
Dicamptodon tenebrosus	Coastal Giant Salamander	Ambystomatidae	1	0	0	0	0	1	0	0	0	0	0	0
Rhyacotriton cascadae	Cascades Torrent Salamander	Rhyacotritonidae	0	0	0	0	0	0	0	0	0	0	0	1
*Rhyacotriton kezeri**	Columbia Torrent Salamander	Rhyacotritonidae	0	0	0	0	0	0	0	0	0	0	0	1
*Rhyacotriton olympicus**	Olympic Torrent Salamander	Rhyacotritonidae	1	0	0	0	0	0	0	0	0	0	0	0
*Rhyacotriton variegatus**	Southern Torrent Salamander	Rhyacotritonidae	1	0	0	0	0	0	0	0	0	0	0	1
Rana aurora	Red-legged Frog	Ranidae	1	0	0	0	0	0	0	0	0	0	0	1
Rana boylii	Foothill Yellow-legged Frog	Ranidae	1	0	0	0	0	0	0	0	0	0	0	1
Batrachoseps wrighti	Oregon Slender Salamander	Plethodonitidae	1	0	0	0	0	0	0	0	0	0	0	1
Plethodon dunni	Dunn's Salamander	Plethodonitidae	1	0	0	0	0	0	0	0	0	0	0	1
Pseudacris regilla	Pacific Tree Frog	Hylidae	1	0	0	0	0	0	0	0	0	0	0	0
Lithobates catesbeianus	American Bullfrog	Ranidae	1	0	0	0	0	0	0	0	0	0	0	1
Pacifastacus leniusculus	Signal Crayfish	Astacidae	1	1	1	1	0	0	0	0	0	0	0	1
Procambarus clarkii	Red swamp Crayfish	Cambaridae	0	0	0	0	0	0	0	0	0	0	0	1
Faxonius rusticus	Rusty Crayfish	Cambaridae	1	0	0	0	0	0	0	0	0	0	0	1
Anodonta oregonensis	Oregon Floater	Unionidae	0	0	0	0	0	0	0	0	0	0	0	1
Gonidea angulata	Western Ridged Mussel	Unionidae	1	0	0	0	0	0	0	0	0	0	0	1
Margaritifera falcata	Western Pearlshell Mussel	Margaritiferidae	1	0	0	0	0	0	0	0	0	0	0	1
Castor canadensis	North American Beaver	Castoridae	1	0	0	0	0	0	0	0	0	0	0	1