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# Whispers of wildfire: using eDNA metabarcoding to decipher effects of megafires on watershed biodiversity

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## Abstract

**Background** Wildfire can dramatically influence terrestrial landscapes, but effects on biodiversity can be hidden beneath the water's surface. Following the 2020 Labor Day wildfires in Oregon's western Cascade Mountain Range, we sampled 25 Oregon streams and 6 Washington streams representing a gradient of fire severity and pre-fire forest stand age to determine the effects of megafires on watershed biodiversity. In each watershed we used environmental DNA (eDNA) metabarcoding and traditional field surveys (Surber or electrofishing) to assess biodiversity.

**Results** Our results suggest aquatic taxonomic richness for macroinvertebrates and vertebrates in the first-year post-fire was unaffected by wildfire. Diversity responses to wildfire severity and pre-fire forest stand age were inconsistent across methods indicating decreasing diversity with increasing burn severity from electrofishing and decreasing diversity with increasing stand age from eDNA, but no reciprocal method changes were observed. eDNA aquatic insect detections revealed greater species richness in older forests (> 90 years). eDNA detected 158 organisms, 48 of which were found in all fire severity categories, but each category revealed unique species compositions.

**Conclusions** We demonstrate the utility of eDNA metabarcoding for a broader taxonomic assessment of post-wildfire disturbance facilitating an improved understanding of the effects of wildfire and forest management on watershed biodiversity.

**Keywords** Environmental DNA, Biodiversity assessment, Megafire, eDNA metabarcoding, Fire Ecology, Metagenomics, Ecological restoration, Pyrodiversity

## Resumen

**Antecedentes** Los fuegos de vegetación pueden influenciar dramáticamente los paisajes terrestres, aunque sus efectos pueden quedar escondidos debajo de la superficie del agua. Luego de los incendios del día del trabajo en las Montañas Cascades del oeste de Oregón, muestreamos 25 arroyos en Oregón y 6 en Washington, que representaban un gradiente de severidad del fuego y la edad de los rodales, para determinar los efectos de los mega incendios sobre la biodiversidad de las micro-cuencas de cada arroyo. En cada una de ellas usamos el código metabar de ADN ambiental (eDNA) y relevamientos de campo (Surber o *electrofishing*), para determinar biodiversidad.

**Resultados** Nuestros resultados sugieren que la riqueza taxonómica acuática para macro invertebrados y vertebrados no fue afectada en el primer año post fuego. La respuesta de la diversidad a la severidad de los incendios y a la edad de los rodales previos al fuego fueron inconsistentes con los métodos usados, indicando un decrecimiento de la

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diversidad con incrementos en la severidad mediante *electrofishing,* y decreciendo en diversidad con un incremento en la edad de los rodales mediante el eADN, pero no fueron encontrados cambios recíprocos entre los métodos usados. Las detecciones de insectos acuáticos mediante el eADN mostró una mayor riqueza de especies en rodales antiguos (> 90 años). El eADN detectó 158 organismos, 48 de los cuales fueron encontrados en todas las categorías de severidad, aunque cada categoría reveló composiciones de especies únicas.

**Conclusiones** Demostramos la utilidad del código eADN metabar para una determinación amplia de los disturbios post fuego, facilitando un mejor entendimiento de los efectos de los fuegos y el manejo forestal en la biodiversidad de arroyos.

## Background

The pyrodiversity-biodiversity hypothesis states that fire can create habitat complexity that promotes and maintains biodiversity in fire-adapted landscapes (Martin and Sapsis 1992; Jones and Tingley 2022), but it has not been evaluated for aquatic assemblages. Freshwater ecosystems are among the most diverse on Earth, representing 10% of all species while covering < 1% of the Earth's surface (Dudgeon et al. 2006), but freshwater populations have declined 83% from 1970 to 2014 at four times the rate of terrestrial populations (Reid et al. 2019). Despite the importance, imperiled status, and excessive loss of aquatic species, research remains biased toward terrestrial organisms (Clark and May 2002; Dudgeon et al. 2006; Darwall et al. 2011; Di Marco et al. 2017; Reid et al. 2019). Global calls to action have documented numerous challenges and stressors for freshwater biodiversity, but none have explicitly included wildfire as a key stressor on freshwater biodiversity (Tickner et al. 2020; Albert et al. 2021; Harper et al. 2021). Stream vertebrate and invertebrate assemblages can serve as indicators of ecological change with shifts in composition (e.g., increased percentage of tolerant taxa) observed in response to stressors such as pollutants, loss of physical habitat complexity, or alterations in watershed hydrology (Whittier et al. 2007; Lücke and Johnson 2009; Hellawell 2012) but have seldom been evaluated in response to wildfire.

Wildfire can alter the structure and function of riparian and freshwater ecosystems by influencing a suite of physical and biological habitat variables that control stream vertebrate populations (Minshall et al. 1989; Mihuc and Minshall 2005; Pettit and Naiman 2007). Fire can induce changes in inputs of wood, sediment, ash, nutrients, organic material, and the stream thermal regime (Koontz et al. 2018; Coble et al. 2023). Fire-driven changes in habitat can influence stream food webs (Spencer et al. 2003; Cooper et al. 2015) and affect stream vertebrates ranging from extirpations to increases in abundance (Dunham et al. 2003; Rieman et al. 2012; Cooper et al. 2015; Swartz et al. 2025). Additionally, wildfires followed by high-precipitation events or post-fire forest management activities (e.g., salvage harvest with replanting) may further alter watersheds with combined effects of multiple disturbances on aquatic biodiversity (Burton 2005; Silins et al. 2014; Gomez Isaza et al. 2022). Wildfires are expected to increase in frequency, size, intensity, and season lengths in many regions including the Pacific Northwest, USA (PNW; Jolly et al. 2015; Abatzoglou et al. 2021; Jones et al. 2022), raising concern for conservation of aquatic and watershed biodiversity in fire-affected systems.

Broad-scale evaluations of aquatic biota responses to wildfire and other landscape conditions are necessary to understand potential effects of anthropogenic and natural disturbances on public and private forest lands. Across the USA, forest practices are monitored for their effectiveness at meeting intended environmental outcomes from implementing federal or state regulations and third-party forest sustainability certification programs (e.g., Sustainable Forestry Initiative (SFI) and Forest Stewardship Council (FSC)). Many state and federal policies focus on water quality and fish habitat conditions as a proxy for protecting a broader suite of aquatic biota, including for USA Endangered Species Act (ESA) listed salmonids (i.e., coho salmon (*Oncorhynchus kisutch*), steelhead (*O. mykiss*), and bull trout (*Salvelinus confluentus*) in the Pacific Northwest, USA. This emphasis may overlook other critical ecosystem responses, including biodiversity, stream assemblage, and less commonly evaluated taxa (e.g., amphibians, crayfish, and non-salmonid fishes) (Dunham et al. 2003; Neville et al. 2009; Swartz et al. 2025). Therefore, biodiversity metrics and emerging sampling tools that examine a broad range of taxa may be more comprehensive indicators of ecosystem responses to forest management practices and other disturbances (Penaluna et al. 2026).

As large-scale wildfires become more frequent and severe in many regions, there has been emerging interest in environmental deoxyribonucleic acid (eDNA) methods to assess wildfire effects on aquatic ecosystems. Organisms shed genetic material into the environment via sloughing of skin, feces deposits, mucus secretions, and reproductive behaviors. Once collected, eDNA can then

be analyzed to identify which species are detected in the collected sample. eDNA is adept at determining presence of species in low abundance without adversely affecting organisms, which can occur with traditional approaches that physically capture individuals. Cryptic aquatic species, including many macroinvertebrates and sculpin species (*Cottus* spp.), cannot be distinguished visually but can be detected and identified based on their genetic signature with eDNA methods (e.g., Penaluna et al. 2023). Multi-species eDNA metabarcoding can capture and characterize the signal of eDNA across broad taxonomic groups and provide a comprehensive assessment of entire ecosystem biodiversity. There are two ways to capture information from multiple species with eDNA metabarcoding (1) by targeting hypervariable regions of mitochondrial genes that are highly conserved across broad taxonomic groups, such as 12S and 16S ribosomal DNA, where a single universal primer pair can amplify multiple species and (2) by using a platform like the Fluidigm 48.48 Access Array™ (Fluidigm©, San Francisco, CA, USA; now called Standard Biotools, 2022) that allows researchers to probe a single sample with multiple primers that target a variety of species with more refined species targets. Multi-gene eDNA metabarcoding from water samples has been previously validated to assess aquatic species in streams in the Pacific Northwest (Hauck et al. 2019; Weitemier et al. 2021; Penaluna et al. 2023), and the use of eDNA to detect terrestrial vertebrates was reviewed by Newton et al. 2025). eDNA metabarcoding with a universal 12S targeting strategy has been shown to detect up to 82% of mammalian species expected to occur in the area (Broadhurst et al 2021) and a variety of mammals, birds, and amphibians from both artificial and natural semi-permanent freshwater sources in Australia (McDonald et al 2023). In the context of wildfire, eDNA has primarily been used for targeted approaches on single species to evaluate their response to fire. For example, wildfire-related single species eDNA approaches were used to examine responses of *O. mykiss* and coastrange sculpin (*C. aleuticus*) to postfire debris flows in California (Rundio et al. 2024), to assess post-fire presence of the myxozoan parasite (*Ceratonova shasta*) after wildfire in California (Richey et al. 2020), to detect a native floater mussel (*Anodonta californiensis/Nuttalliana)* after wildfire (Lawrence et al. 2022), and to track impact of megafires on platypus (*Ornithorhynchus anatinu*) occupancy in Australia (McColl-Gausden et al 2023). These prior studies demonstrate the usefulness of eDNA to monitor the effects of fire on stream inhabitants. Australian researchers are beginning to use eDNA-derived amplicon sequence variants to examine community structure and shifts in community structure as a function of fire (Bracewell et al. 2023). This method differs from eDNA metabarcoding in that the actual species detected are not identified to the operational taxonomic unit (OTU) or species level and is mostly used for microbial communities. To our knowledge, no studies have used stream sourced eDNA with metabarcoding to comprehensively inventory biodiversity of watershed communities after wildfire across a range of fire severities. By simultaneously considering assemblages across multiple taxonomic groups, we can better understand the full effects of wildfire on aquatic organisms.

The links among characteristics of forested watersheds (e.g., forest stand age), fire severity, and aquatic biodiversity are unclear, ultimately hindering our ability to prioritize actions that would promote the resiliency of aquatic biodiversity in forests from fire disturbance. Thus, our primary objective was to evaluate relationships of aquatic and watershed biological diversity with pre-fire forest management, fire severity, or their interaction. We used a space-for-time approach to disentangle these effects with stratified random sampling of forested watersheds which span a gradient of pre-fire forest stand age and watershed burn severity. Using eDNA and traditional survey techniques, we evaluated the aquatic and watershed biodiversity 1-year post-fire at burned sites in Oregon across a fire severity gradient and at unburned reference watersheds in Oregon and Washington to assess the utility of eDNA as a tool to measure post-fire biodiversity responses. We also apply two eDNA data analysis tools, eDNAJoint (Keller 2025, an R package that uses a Bayesian modeling framework to analyze eDNA data with paired or semi-paired traditional surveys) and Ranacapa (Kandlikar, 2018, a web application using R and Shiny to facilitate the analysis and visualization of eDNA data), and demonstrate how they can be used to advance the use of eDNA metabarcoding for broad taxonomic assessments following disturbances from wildfires or other events. These efforts simultaneously allowed us to characterize the local distribution of species with regionally elevated conservation status of critically imperiled, imperiled and vulnerable, USA Endangered Species Act Threatened, and specifically defined priority species for managed forests in the Pacific Northwest.

## Methods

### Study sites and characteristics

In September 2020, approximately 11% of Oregon's Cascade Ecoregion burned in the Labor Day Fires (Abatzoglou et al. 2020; Reilly et al. 2022) including the Riverside, Beachie Creek, and Holiday Farm complexes which collectively burned 2043.7 $km^2$. These fires burned at varying severities, which resulted in a range of post-fire conditions across landscapes. Within the first post-fire year (summer 2021), traditional and eDNA sampling methods were used to assess aquatic biodiversity

responses to wildfire in Oregon at 25 sites in 4th order streams that encompassed a range of pre-fire stand age and fire severity. Fire severity was assessed for each whole watershed as mean basal area mortality (BAM %); basal area mortality values are initial assessments of burn severity provided by the Rapid Assessment of Vegetation Condition after Wildfire (RAVG) program from the U.S. Department of Agriculture Forest Service Geospatial Technology and Applications Center (https://burnseverity.cr.usgs.gov/ravg/data-access) and averaged for each watershed. The "highly" burned sites ($n = 8$) ranged from 30 to 88% BAM, the "moderately burned" sites ($n = 9$) had 6 to 28% BAM and the "unburned" reference sites near/within the fire boundaries ($n = 8$) had BAM < 1%. We also collected samples at 4th order streams in Washington's western Cascade Mountain Range ($n = 6$) which had a similar mean forest stand age and elevation to the Oregon sites to expand the scope and conditions within the unburned reference groups (see Fig. 1 for a map of all sample sites). Site selection followed that in Coble et al. (2023) using a stratified random sampling design of pre-fire stand age and fire severity to select 25 4th order watersheds (Strahler 1957) within 6 km of each of the three fire boundaries. For each site, the following characteristics were summarized by the most-downstream sampling point: pre-fire forest stand age (< 85 or > 85 years based on 2017 Landscape, Ecology, Modeling, Mapping, and Analysis (LEMMA) layer), percent of watershed burned, BAM %, and soil burn severity (obtained from the Burned Area Emergency Response (BAER) database) within the riparian-management areas (RMA) defined as the area within 30.48 m (100 ft) of the stream with values of unburned, low and moderate severity (Table 1). LEMMA was used to assess pre-fire forest stand age using a previously developed and validated remote sensing layer based on Landsat imagery (30 m resolution) available at https://lemma.forestry.oregonstate.edu/data/structure-maps (Bell et al. 2015; Davis et al. 2015; Ohmann et al. 2012; Ohmann and Gregory 2002). While fire can also contribute to forest stand age, we did not find any major fires within our study watershed boundaries to suggest fire has shaped stand ages more recently (1900–2019, https://hub.oregonexplorer.info/datasets/OSUGISci::historic-fires-pre-2000/about), so we attribute stand age to forest management practices. Soil burn severity obtained from the BAER database available at https://burnseverity.cr.usgs.gov/baer/baer-imagery-support-data-download, combines satellite-derived imagery analysis and field validation for classifications.

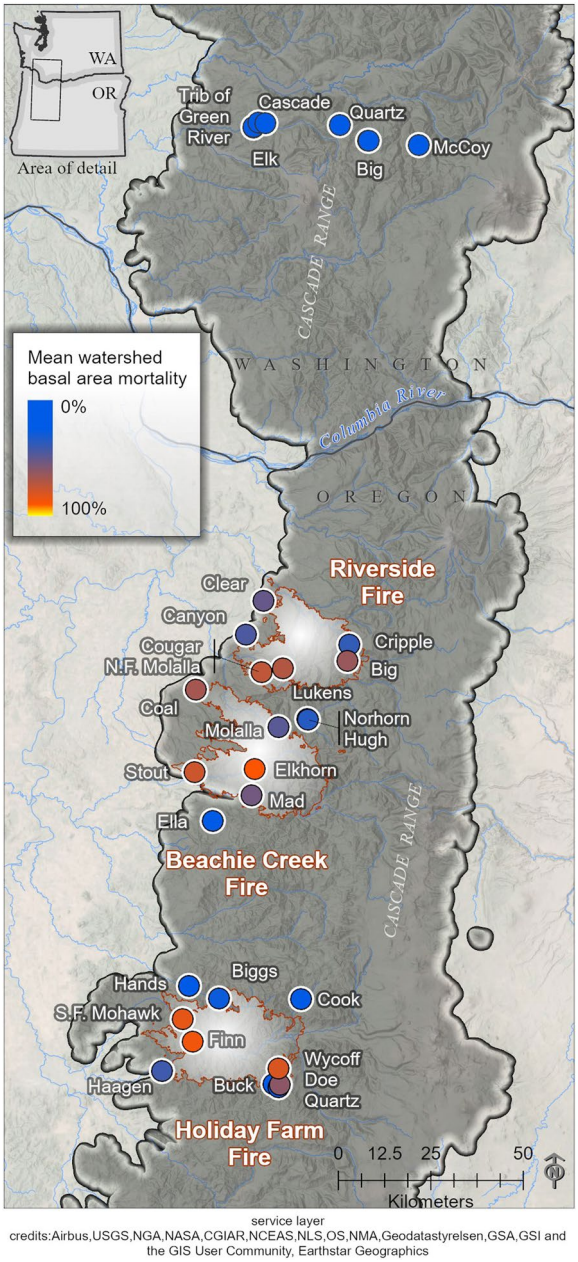



**Fig. 1** Sampling sites in the Cascade Mountain Range (dark grey shading) for the twenty-five 4th-order stream sites in Oregon and six 4th-order stream reference sites in Washington. The color of the circles indicates the mean basal area mortality (BAM, %) in percentage of area with blue circles indicating unburned reference sites (less than 1% BAM) and purple to red colors indicating greater BAM. Boundaries of three of the wildfires from the Labor Day fires in 2020 are represented by red outlines

### Traditional sampling

Traditional sampling for vertebrates and macroinvertebrates was conducted on the same day and immediately after eDNA samples were taken (to limit contamination) following the procedures as described in (Coble et al. 2023 and Swartz et al. 2025). Triple-pass depletion backpack electrofishing was used to capture, identify (based on visual characteristics), count, and measure lengths and

**Table 1** Site information for all locations including GPS coordinates, mean pre-fire stand age, what type of data was collected for each site, mean watershed basal area mortality % (BAM), BAM burn severity category, soil burn severity category (RMA), percent of watershed burned, and associated fire complex

| Stream/river name | GPS coordinates | Mean pre-fire stand age (y) | eDNA | Electrofishing | Surber | Watershed basal area mortality % (BAM) | BAM burn severity category | Soil burn severity category (RMA) | Percent of watershed burned | Associated fire complex |
|---|---|---|---|---|---|---|---|---|---|---|
| Oregon sites | | | | | | | | | | |
| Big | 45° 04′ 09.0″ N 122° 05′ 11.0″ W | 146 | ✓ | ✓ | ✓ | 28% | Low | Low | 100% | Riverside |
| Biggs | 44° 17′ 14.0″ N 122° 36′ 51.0″ W | 62 | ✓ | ✓ | ✓ | 0% | Unburned | Unburned | 0% | Holiday |
| Buck | 44° 02′ 44.4″ N 122° 19′ 52.6″ W | 160 | ✓ | ✓ | ✓ | 0% | Unburned | Unburned | 0% | Holiday |
| Canyon | 45° 08′ 31.3″ N 122° 24′ 46.5″ W | 72 | ✓ | ✓ | ✓ | 13% | Low | Unburned | 32% | Riverside |
| Clear | 45° 12′ 33.9″ N 122° 20′ 32.1″ W | 84 | ✓ | ✓ | ✓ | 21% | Low | Unburned | 78% | Riverside |
| Coal | 45° 00′ 37.7″ N 122° 35′ 15.7″ W | 59 | ✓ | ✓ | ✓ | 30% | High | Moderate | 92% | Beachie creek |
| Cook | 44° 15′ 11.7″ N 122° 14′ 03.8″ W | 194 | ✓ | ✓ | ✓ | 0% | Unburned | Unburned | 0% | Holiday |
| Cougar | 45° 03′ 06.8″ N 122° 21′ 36.3″ W | 83 | ✓ | ✓ | ✓ | 24% | Low | Low | 100% | Riverside |
| Cripple | 45° 06′ 59.2″ N 122° 03′ 28.4″ W | 189 | ✓ | ✓ | ✓ | 6% | Low | Moderate | 24% | Riverside |
| Doe | 44° 02′ 39.0″ N 122° 18′ 36.2″ W | 81 | ✓ | ✓ | ✓ | 26% | Low | Low | 65% | Holiday |
| Elkhorn | 44° 48′ 54.0″ N 122° 23′ 11.1″ W | 140 | ✓ | ✓ | ✓ | 88% | High | Moderate | 100% | Beachie creek |
| Ella | 44° 41′12.8″ N 122° 31′ 47.1″ W | 77 | ✓ | ✓ | ✓ | 0% | Unburned | Unburned | 0% | Beachie creek |
| Finn | 44° 09′ 04.8″ N 122° 36′ 08.4″ W | 68 | ✓ | ✓ | ✓ | 80% | High | Moderate | 100% | Holiday |
| Haagen | 44° 04′ 47.9″ N 122° 42′ 26.5″ W | 45 | ✓ | ✓ | ✓ | 10% | Low | Low | 36% | Holiday |
| Hands | 44° 15′ 23.0″ N 122° 30′ 49.5″ W | 74 | ✓ | ✓ | ✓ | 0.003% | Unburned | Unburned | 0.5% | Holiday |
| Hugh | 44° 56′ 06.0″ N 122° 12′ 06.9″ W | 168 | ✓ | ✓ | ✓ | 0.2% | Unburned | Unburned | 9% | Beachie creek |
| Lukens | 45° 03′ 38.2″ N 122° 17′ 09.7″ W | 157 | ✓ | ✓ | ✓ | 42% | High | Low | 100% | Riverside |
| Mad | 44° 44′ 20.8″ N 122° 23′ 04.5″ W | 82 | ✓ | ✓ | ✓ | 23% | Low | Low | 79% | Beachie creek |
| Molalla River | 44° 54′ 59.8″ N 122° 18′ 09.5″ W | 117 | ✓ | ✓ | ✓ | 16% | Low | Low | 46% | Beachie creek |

**Table 1** (continued)

| Stream/river name | GPS coordinates | Mean pre-fire stand age (y) | eDNA | Electrofishing | Surber | Watershed basal area mortality % (BAM) | BAM burn severity category | Soil burn severity category (RMA) | Percent of watershed burned | Associated fire complex |
|---|---|---|---|---|---|---|---|---|---|---|
| N. Fork Molalla River | 45° 03′ 03.1″ N 122° 21′ 37.0″ W | 80 | ✓ | ✓ | ✓ | 44% | High | Low | 87% | Riverside |
| Norhorn | 44° 56′ 09.4″ N 122° 12′ 20.9″ W | 174 | ✓ | ✓ | ✓ | 0.1% | Unburned | Unburned | 4% | Beachie creek |
| Quartz | 44° 02′ 21.1″ N 122° 18′ 50.1″ W | 164 | ✓ | ✓ | ✓ | 0% | Unburned | Unburned | 0% | Holiday |
| S. Fork Mohawk River | 44° 12′ 22.8″ N 122° 38′ 11.8″ W | 58 | ✓ | ✓ | ✓ | 60% | High | Low | 100% | Holiday |
| Stout | 44° 48′ 32.9″ N 122° 35′ 29.5″ W | 81 | ✓ | ✓ | ✓ | 47% | High | Moderate | 100% | Beachie creek |
| Wycoff | 44° 05′ 02.7″ N 122° 18′ 51.5″ W | 83 | ✓ | ✓ | ✓ | 55% | High | Low | 92% | Holiday |
| Washington reference sites | | | | | | | | | | |
| Big | 46° 20′ 39.6″ N 121° 58′ 12.1″ W | 141 | ✓ | | ✓ | | | | | |
| Cascade | 46° 23′ 25.8″ N 122° 21′ 20.7″ W | 47 | ✓ | | ✓ | | | | | |
| Elk Creek 1 West | 46° 23′ 22.1″ N 122° 19′ 50.8″ W | 53 | ✓ | | ✓ | | | | | |
| Green River Tributary | 46° 22′ 46.7″ N 122° 22′ 25.6″ W | 67 | ✓ | | ✓ | | | | | |
| McCoy | 46° 19′ 52.3″ N 121° 47′ 32.8″ W | 116 | ✓ | | ✓ | | | | | |
| Quartz | 46° 23′ 00.8″ N 122° 04′ 08.3″ W | 83 | ✓ | | ✓ | | | | | |

widths of all vertebrates (fish and stream-living amphibians) and crayfish caught with this approach (Hanks et al. 2018). Two 20-m sections were separated with block nets for a 40 m sampling reach. Collected fish and amphibians were placed in an aerated bucket for recovery prior to being released in the reach where they were captured. A Surber sampler was used (0.09 $m^2$, 43 µm mesh) to quantify macroinvertebrates. At each of 3 locations (0, 40, 80 m) within each stream reach, a Surber sampler was placed on the streambed facing upstream. Substrate was disturbed to a depth of 10 cm for 2 min, and large rocks were scrubbed within the sample and then organisms from all three locations were composited into a single sample and preserved with 95% ethanol in HDPE sample bottles. Collected macroinvertebrates were sent to a local taxonomic expert to be enumerated and identified to the lowest operational taxon unit (OTU).

#### eDNA sampling/extraction

Eight replicates of 3 L water samples were collected per site (2 L thalweg + 1 L adjacent slack water). To prevent contamination, the sampling location was approached from downstream (0 m) at the bottom of the electrofishing reach. eDNA samples were taken prior to any other measurements or entry into the stream. Bottles and tweezers were decontaminated with a 50% bleach solution followed by a triple rinse of deionized water between sites. Water was filtered and eDNA was extracted as described in Hauck et al. 2019). Briefly, water was filtered streamside through 0.45-µm single-use cellulose nitrate filters (Sterlitech, Kent, WA, USA). Filters were loosely rolled and stored in 5 mL vials on wet ice during collection and transport, frozen within 6 h of collection and stored at −20 °C until DNA extraction. DNA was extracted with the DNeasy PowerWater© 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). DNA was quantified using fluorometry (Qubit™ High Sensitivity kit; ThermoFisher). The targeted eDNA submission concentration was 12–15 ng/µl; however, the concentration of some samples was lower due to insufficient DNA extraction yield.

#### eDNA amplification

Extracted eDNA samples were amplified on a Fluidigm 48.48 Access Array™ (Fluidigm, San Francisco, CA, USA; Fluidigm Corporation, 2016) at the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign using the one-step Fluidigm cycling protocol as described in Hauck et al. 2019). This platform allows targeting of up to 48 unique primers across 48 individual samples in 2304 molecularly barcoded independent reactions per array. The use of lower-resolution universal metabarcoding targets, such as mitochondrial 12S and 16S Ribosomal DNA, was mixed with higher-resolution targets such as cytochrome oxidase I (COI) and cytochrome B (cytB). Targeting both types of genes allowed for detection of a greater number of species without loss of taxonomic resolution. Design of the primers and amplification strategy is further detailed in Hauck et al. (2019). A full list of primers and gene targets used in this study is in Supplemental Table 1. To mitigate PCR inhibition from environmental DNA preparations, bovine serum albumin (BSA) was added at 0.2 µg/µL final volume. Each array included positive and negative controls to assess primer functionality, assist with bioinformatics and monitor for contamination. The positive control contained a mixture of genomic DNA derived from targeted species plus λ bacteriophage DNA (New England Biolabs, Ipswich, MA, USA), each mixed at equal mass and diluted to a final concentration of 10 ng/µl. Negative controls consisted of λ DNA submitted at 10 ng/µl. Amplified barcoded targets were then combined with equal volumes and sequenced on an Illumina NovaSeq 6000 with 2×250 bp paired-end reads. Sequences were demultiplexed by index and by primer, allowing up to one mismatch in the index and two mismatches in the primer, and ϕX174 DNA control sequences were removed at the Roy J. Carver Biotechnology Center. Raw sorted reads have been uploaded into the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) and can be accessed by searching for BioProject Accession Number SUB15183378.

#### Bioinformatics

Sorted reads were processed as described in Hauck et al. (2023) with the exception that read 1 and read 2 were joined into contigs prior to trimming out the primer portions of the read using Trimmomatic v. 0.33. To avoid including primer dimers in our final dataset, a minimum length filter was set at 150 bp and adjusted where evidence of longer primer dimers was detected. The trimmed contigs were then classified to taxon using the programs KMA, version 1.2.23 (Clausen et al., 2018), and CC-Metagen version 1.2.2 (Marcelino et al., 2020) using the Marcelino indexed database dated 11 June 2019 (includes all sequences from the NCBI nucleotide collection as of that date except environmental or artificial sequences). As the data included multiple gene targets of varying conservation, length and taxonomic origin, the CC-Metagen thresholds were modified to better capture species richness. Using sequences from the positive controls to guide parameters, the following taxon threshold settings were determined to be optimal for this data set as they provided the most accurate identities possible in

the positive control sequences across all genes and species targeted: class = 80.91, order = 81.21, family = 88.3, genus = 96.2, and species = 97.8. Barcode swapping (also referred to index hopping) is a phenomenon that occurs on high-throughput sequencers using patterned flow cells such as the Novaseq machine that generated our data (Costello et al., 2018). As described in Flitcroft et al. (2025), barcode swaps show up as a long tail of low-count random detections in the data that account for less than 1% of the total dataset. To mitigate the barcode swaps, the bottom 1% of reads tallied by primer, taxon, and sample were dropped. The 1% minimum read cutoff for this data set was 542. Data were then deduplicated and summed where haplotypes resulted in multiple reports of the same species at a single site and primer combination.

#### Curation of the dataset

Molecular identifications of aquatic insect species were validated by local experts (Gerth 2025). When detected species were not on the list of aquatic insects known to be in Oregon, the identification was adjusted to reflect the next lowest taxonomic level of genus. All aquatic insect amplicons identified at taxonomic levels above species were left as determined by the KMA/CC-Metagen pipeline. All Salmonid *Oncorhynchus* species amplified with universal primers (12S or 16S loci) were reassigned to the genus level due to insufficient barcode gaps at that locus for reliable species identification within that closely related taxonomic group. Species level detections removed from the final dataset include all Oomycota *Phytophthora* due to the need for additional bioinformatic analysis, all bacterial amplicons, non-informative detections (i.e., humans, *Homo sapiens*), and a limited number of non-invasive species out of their geographical range that we have tracked to issues with the database. Finally, with the exception of aquatic insect OTUs, any organism identified above the level of species was removed from the final dataset to avoid ambiguity in our comparisons with traditionally collected data.

#### Data analysis/statistics

The eDNA detections in the final dataset were summed and averaged per replicate per site and grouped for comparison with traditional data as follows:

1) eDNA electrofishing species: eDNA detections were filtered to include species that can also be captured by traditional electrofishing methods in these Oregon sites: fish, amphibians, and crayfish (see Supplemental Fig. 1 for heatmap of eDNA detections in this category).
2) eDNA aquatic insect OTUs: detections of all aquatic insects identified at the lowest taxonomic level. This included detections from order, family, genus, and species (see Supplemental Fig. 2 for Krona capturing all aquatic insect eDNA detections).
3) eDNA all other species: detections of all Oregon species level eDNA detections except for aquatic insects. This included fish, amphibians, crayfish, mammals, birds, terrestrial insects and worms (see Supplemental Fig. 3) for heatmap of eDNA detections in this category).
4) eDNA watershed biodiversity: detections included all eDNA detections as defined above for *eDNA aquatic insect OTUs* and *eDNA all other* species from both Oregon and Washington. This combined group represented biodiversity of the entire watershed as captured by eDNA detections.

The *eDNA electrofishing species* and *eDNA all other species* datasets were rarified (×10 subsampling events) to the minimum number of reads in a single sample (21,261) using the Ranacapa program (Kandlikar et al 2018), and the rarified data was used in the same program to generate Shannon's index values. eDNA Shannon's index (H′) values were used to evaluate effects of pre-fire mean watershed stand-age across the fire gradient and for comparisons with the traditional data diversity index values. Shannon's index values for traditional detections were calculated as:

$$Shannon's\ Index\left(H^{'}\right) = -\sum\left(p_i * \ In(p_i)\right)$$

where $p_i$ is the proportion (n/N) of individuals of the ith species found (n) divided by the total number of individuals found (N), for the number of species present in each sample. Higher values of H indicated a higher species diversity. Aquatic insect data from both methods were assessed at the lowest OTU and explored further as species richness across the fire gradient and mean forest stand age. Following a lack of a significant interaction effect, linear regression and fitted second-order polynomials (where the data were better explained with second versus first-order regressions) were used and we considered significance at $p \leq 0.05$.

The eDNA species-level detections that matched species that were also found by electrofishing were further analyzed using eDNAJoint (Keller 2025). This package interprets paired and semi-paired eDNA and traditional data in a Bayesian framework to better understand the relative sensitivities of the two survey types and explores the role of covariates in eDNA detections. Leave-one-out cross validation was used to compare models with and without the site-level covariates in the model, and the predictive ability of the model is measured with expected log pointwise predictive density (ELPD). In the absence

of documented catch rates at the sampling locations, the expected catch rate (mu) was left at 0.9 for all species in all analyses.

## Results

### Species detection by method

Traditional electrofishing captured 16 species and the Surber surveys captured 184 aquatic insect OTUs. The eDNA amplicon sequencing run produced ~336 million (M) paired reads from 48 different primers targeting fishes, amphibians, invertebrates, *Phytophthora*, mammals, and animal pathogens. Of those, ~257 M reads passed quality filtering, pairing, and were identified to taxa, and ~161 M reads passed minimum read threshold filtering of 542 reads and were sorted to this project (not including the positive controls). There were 262 organisms with unique taxonomic identities (taxIDs) identified in field samples using eDNA metabarcoding (including all organisms at all taxonomic levels of detection). After curation, the final eDNA Watershed Biodiversity dataset contained 158 organisms, 113 of which were aquatic insect OTUs.

### Aquatic insect response to fire

*eDNA aquatic insect OTUs* revealed greater species richness in older forests (> 90 years, $p = 0.001$) while not showing a significant response to burn severity (BAM%; $p = 0.2$), and Surber survey results revealed no significant variation in relationship to burn severity (BAM%; $p = 0.2$) or pre-fire forest stand age ($p = 0.46$, Fig. 2a and b). Although only significant for one method, both methods showed similar quadratic trends to both predictors despite sharing only 30 OTUs between the methods. eDNA failed to detect insects from the order *Trichoptera* (caddisflies) while Surber surveys captured 38 OTUs, and eDNA detected 13 OTUs from the order *Diptera* (flies) while Surber surveys captured 85 *Diptera* OTUs. These discrepancies account for the large numerical difference between the methods with Surber survey counting 71 more aquatic insect OTUs than eDNA detected (see Supplemental Fig. 2 and Supplemental Fig. 4 for Krona graphs of all aquatic insect detections by eDNA and Surber surveys respectively).

### All other species (excluding aquatic insects) response to fire

Our regression analysis found no significant interaction of mean pre-fire forest stand age with fire severity (BAM %) in predicting species richness or Shannon's diversity for eDNA (filtered for electrofishing species only (fish, amphibians, and crayfish) and as a comprehensive dataset) or electrofishing. However, we identified patterns that revealed subtle shifts in response to both factors separately, though with low $R^2$ values indicating that the patterns do not fully explain the variance observed. With Shannon's Index, electrofishing data showed an inverse relationship ($p <= 0.01$) to watershed burn severity in contrast to the lack of a significant response found with eDNA electrofishing species ($p = 0.3$) and eDNA all other species ($p = 0.25$) which weakly increased with increasing BAM % (Fig. 3c). Electrofishing did not vary with pre-fire forest stand age ($p = 1.0$), whereas rarified eDNA Shannon's values for eDNA electrofishing species ($p = 0.06$) and eDNA all other species ($p = 0.04$) decreased with increasing stand age (Fig. 3d). We found no significant interaction of mean fire severity (BAM %) or pre-fire forest stand age respectively in predicting species richness for electrofishing ($p = 0.41$, $p = 0.96$), eDNA

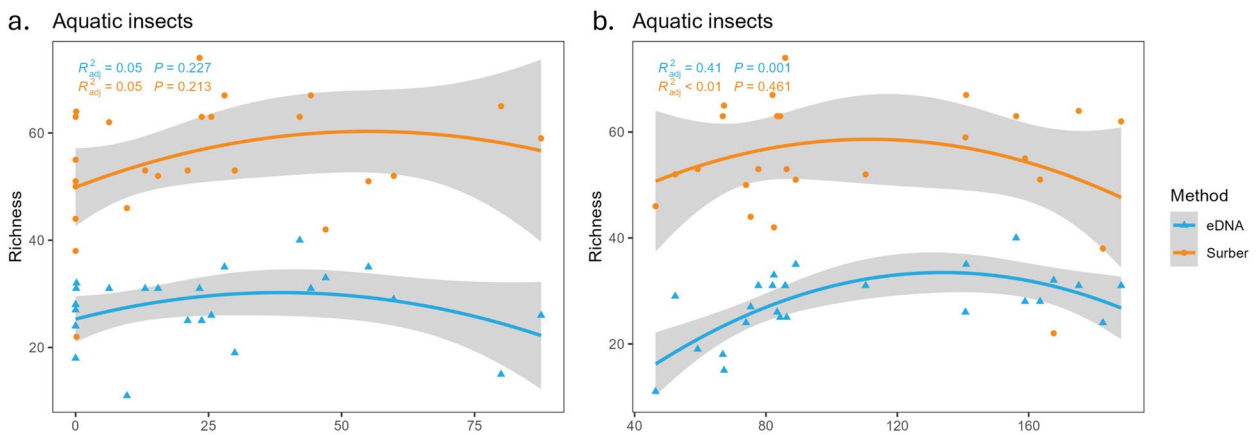



**Fig. 2** Species richness for aquatic insects from both eDNA and Surber survey methods as a function of watershed burn severity assessed by mean watershed basal area mortality (BAM %) (**a**) and mean watershed stand age in years (**b**). Aquatic insects data consist of insects from the following orders: Ephemeroptera, Plecoptera, Coleoptera, Hemiptera, Neuroptera, Odonata, Diptera, Trichoptera, Hydracarina, and Megaloptera

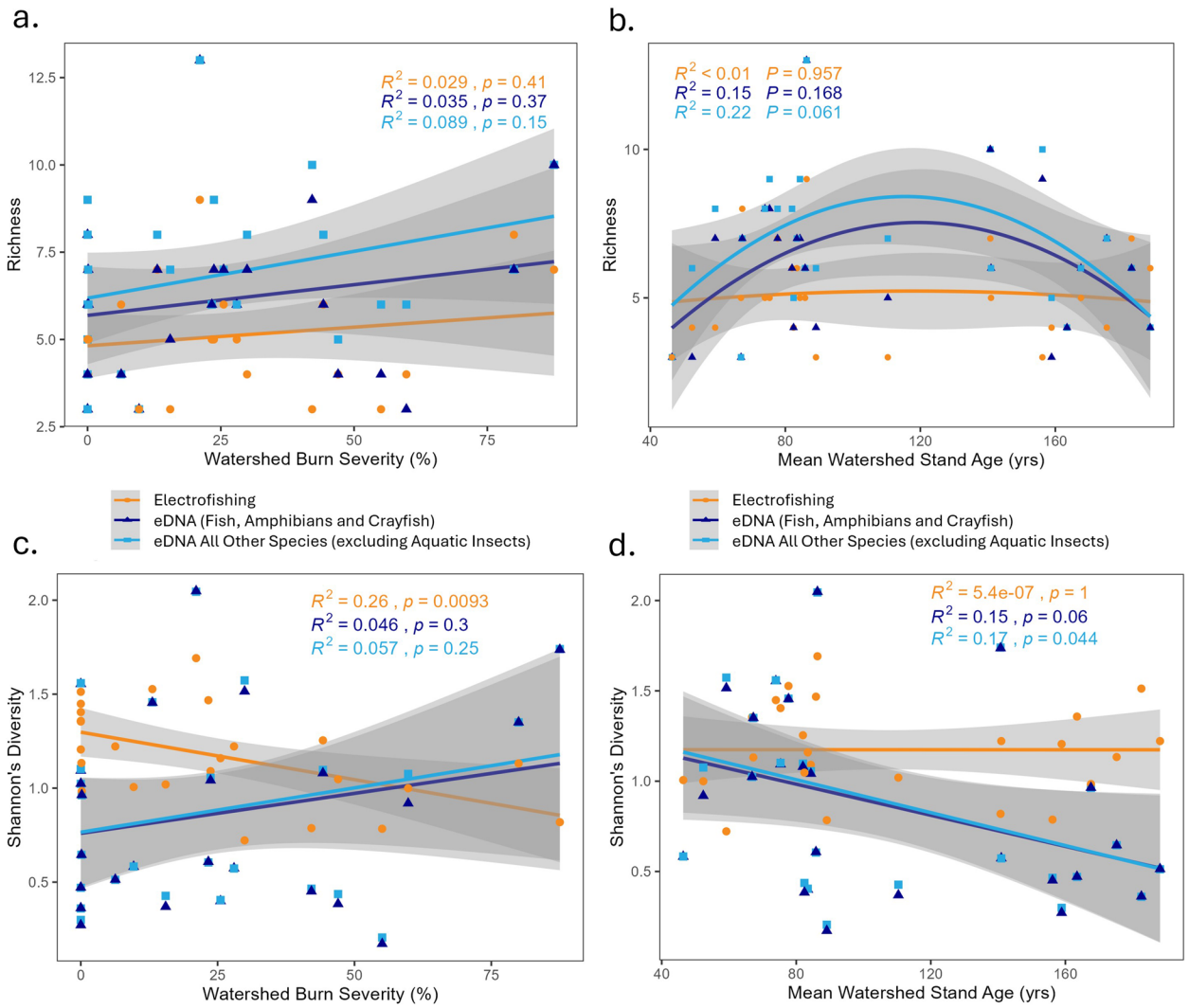



**Fig. 3** Species richness from both eDNA and electrofishing as a function of watershed burn severity (BAM %) (**a**) and mean watershed stand age (**b**). Shannon's diversity index for both rarefied eDNA and electrofishing as a function of watershed burn severity (BAM%) (**c**) and mean watershed stand age (**d**). The eDNA data is presented in two ways, filtered for electrofishing species only (fish, amphibians, and crayfish) and as a comprehensive dataset of all detections excluding aquatic insects

electrofishing species ($p = 0.37$, $p = 0.17$), or eDNA all other species ($p = 0.15$, $p = 0.06$) (Fig. 3a and b).

### Comparison of eDNA and electrofishing methods

The eDNA data filtered to remove the aquatic insect OTUs and the Washington detections (where no electrofishing occurred) resulted in 40 "all other species" detections for the eDNA dataset. Of these 40 species, 25 were detected only with eDNA, 15 were shared detections with electrofishing, and 1 species (Cascades frog, *Rana cascadae*) was found only by electrofishing (Fig. 4). The shared detections included coho salmon (*O. kisutch*), Chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*Oncorhynchus mykiss*), coastal cutthroat trout (*Oncorhynchus clarkii clarkii*), brook trout (*Salvelinus fontinalis*), Pacific lamprey (*Entosphenus tridentatus*), Western river lamprey (*Occidentis richardsoni*), torrent sculpin (*Cottus rhotheus*), longnose dace (*Rhinichthys cataractae*), speckled dace (*Rhinichthys osculus*), coastal tailed frog (*Ascaphus truei*), Pacific chorus frog (*Pseudacris regilla*), red-legged frog (*Rana aurora*), coastal giant salamander (*Dicamptodon tenebrosus*), and signal crayfish (*Pacifastacus leniusculus*). The molecular resolution of eDNA allowed us to detect 5 additional lineages of *Cottus* sculpin that were unable to be identified to species in the field (Paiute

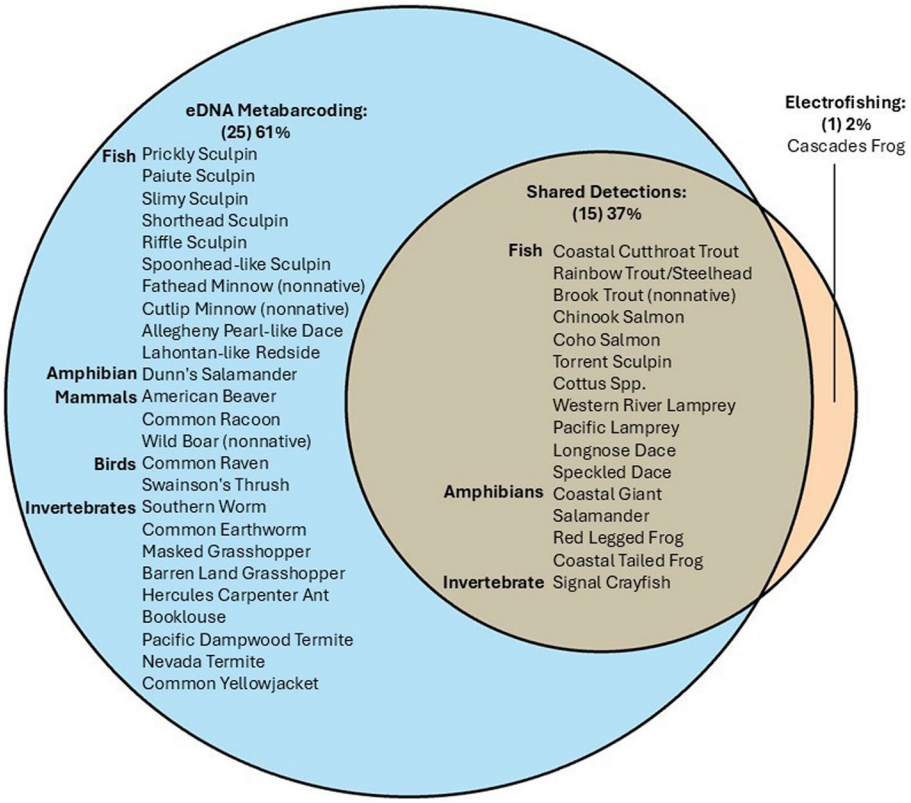



**Fig. 4** Comparison of all species (excluding aquatic insects) detected by eDNA metabarcoding and aquatic species captured by electrofishing in Oregon

sculpin (*C. beldingii*), prickly sculpin (*C. asper*), slimy sculpin (*C. cognatus*), shorthead sculpin (*C. confusus*), riffle sculpin (*C. gulosus*), and spoonhead-like sculpin (*C. ricei*). The young-of-the-year trout cannot be phenotypically identified as either coastal cutthroat trout or rainbow trout with traditional methods, but can be genetically identified with eDNA data. eDNA also detected some additional nonnative minnow species not captured with electrofishing and terrestrial species including: Dunn's salamander (*Plethodon dunni*), birds (Swainson's thrush (*Catharus ustulatus*) and common raven (*Corvus corax*)), terrestrial invertebrates (masked and barren land grasshoppers (*Trimerotropis cincta* and *Trimerotropis pistrinaria*), Hercules carpenter ant (Camponotus herculeanus), booklouse (*Liposcelis decolor*), termites (*Zootermopsis angusticollis* and *Zootermopsis nevadensis*) and yellowjacket bee (*Vespula alascensis*)) and 3 mammals (American beaver (*Castor canadensis*), common racoon (*Procyon lotor*), and invasive wild boar (*Sus scrofa*)). Robustness of eDNA metabarcoding detections (average number of eDNA reads per replicate) roughly correlated with abundance data for species captured by electrofishing (Fig. 5). Salmonid detections were more robust than those for other species, and the eDNA signal for amphibians was the lowest in the eDNA data set.

eDNAJoint, analysis revealed that some species require more replicates for reliable detection when compared to the electrofishing survey, and that fire impacted our ability to detect species in different ways (Fig. 6). For example, with a capture rate (mu) of 0.9, the non-covariate model predicts that we could detect rainbow trout with 0.9 probability with only 3 eDNA field replicates in, but detection of coho salmon in the same model would require 26 replicates. It should be noted that we do not expect coho salmon in these creeks to have a capture rate of 0.9, in fact it was only captured at two locations with electrofishing. This may explain the unexpectedly high eDNA replicate requirement to detect them; however, we do not have documented catch rates in these sampling locations for any of the species, and in the absence of that data, we left mu at the same value, 0.9, for all species at all locations during the analysis. BAM% alone did emerge as a predictive model for coho salmon, coastal tailed frog and signal crayfish. For coho salmon and coastal tailed frog, the negative α2 translates to these species being more likely to be detected and predicts with 0.9 probability that we would detect both coho salmon and coastal

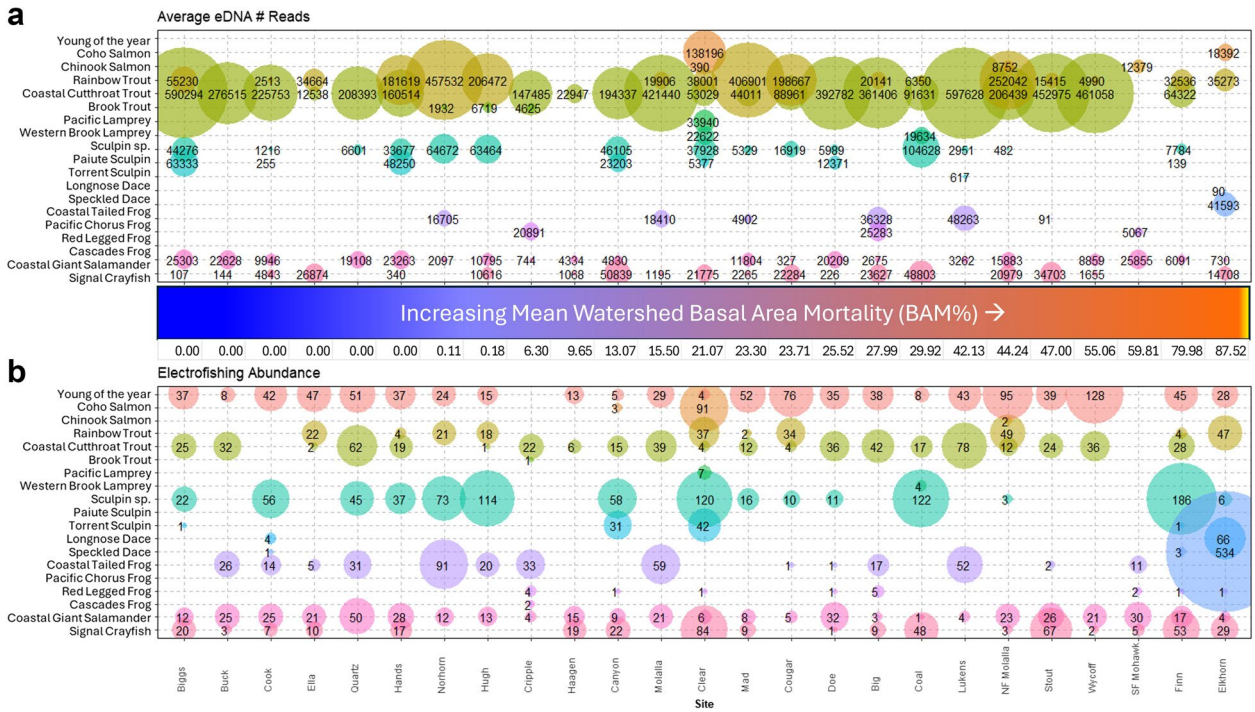



**Fig. 5** Relative abundance of species detected at each site in Oregon by average eDNA reads per replicate per species (**a**) and electrofishing abundance of species captured (**b**). Sites are ordered by mean watershed basal area mortality (BAM%) with unburned sites (blue) through high severity burn sites (red). eDNA species listed are limited to only those species captured by electrofishing, including fishes, amphibians, and crayfish

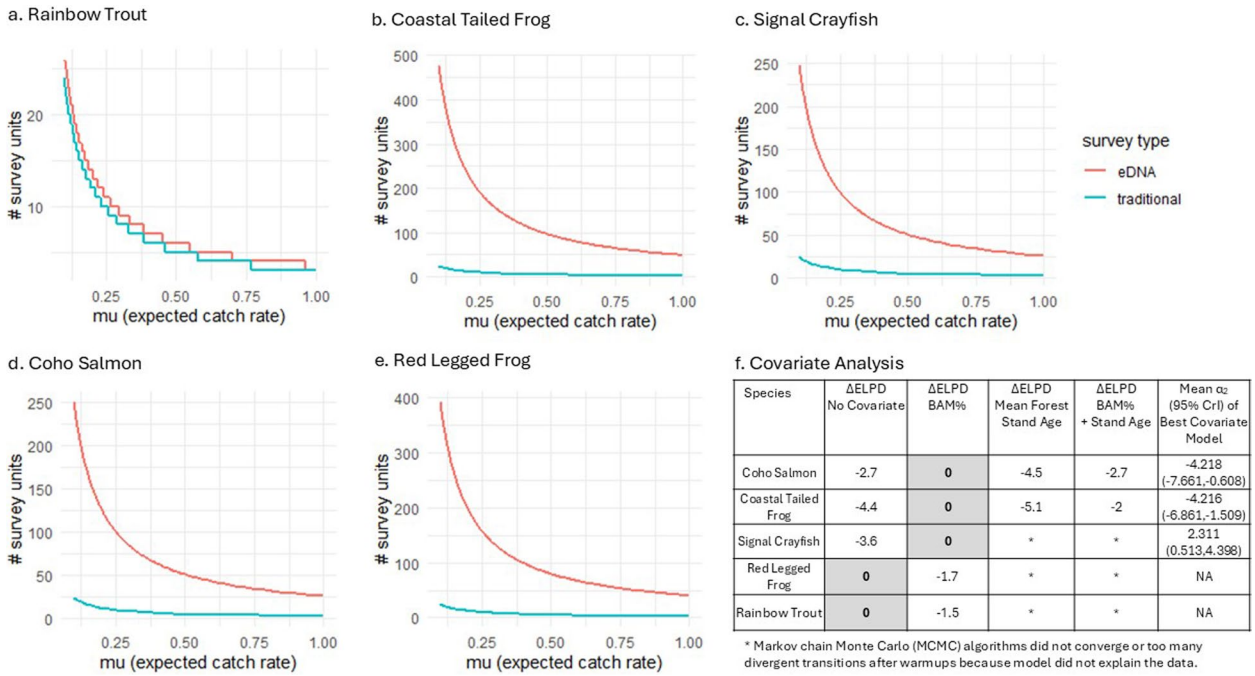
tailed frog with just 2 field eDNA replicates in burned areas, which is a significant improvement over the non-covariate model results. The positive α2 for signal crayfish indicates that this species is more difficult to detect within burned areas and translated to the need for 116 field eDNA replicates up from 26. Mean forest stand age and BAM% combined with mean forest stand age failed in the statistical models in many cases and never came out as the best model to explain the outcome. It should be noted that our data sets were too small or lacking in complexity (species too widely distributed) to successfully model the results for coastal giant salamander (*Dicamptodon tenebrosus*), western river lamprey, cutthroat trout, Chinook salmon, and brook trout (*Salvelinus fontinalis*) resulting in statistical errors.

### Watershed biodiversity

A Venn diagram visualizing the overlap of the 158 eDNA Watershed Biodiversity detections among unburned (Oregon), unburned (Washington), low BAM% burn severity (Oregon), and high BAM% burn severity (Oregon) groups revealed that each category both supported unique species communities and had substantial overlap with other groups (Fig. 7). Forty-eight species (30% of all detections) were represented across all groups of unburned and burned sites. There were 25 species (16%) detections unique to unburned reference sites in Washington (which may be a product of regional differences in species composition), 10 species (6%) unique to the unburned Oregon and low burn severity sites, and 22 species (14%) were found only in watersheds with high burn severity. Of interest, 38 species (24%) were only found in burned locations, with only 6 in both high and moderate burn severities, and 40 species (25%) were only found in unburned locations with only 5 in both Oregon and Washington unburned locations (see Supplemental Table 2 for a list of the unique groupings of species detected).

### Aquatic insect collections

Aquatic insect biodiversity captured by eDNA detected approximately similar numbers of OTUs for Ephemeroptera and roughly 3X more OTUs than from Surber surveys for Plecoptera (Fig. 8); however, eDNA failed to detect Trichoptera (caddisflies) and detected relatively few Diptera. Those taxa represented the majority of OTUs captured by Surber surveys (38 Trichoptera and 85 Diptera—123 of the 184 taxa captured) leading to the traditional survey detecting more species overall (supplemental Fig. 2). Outside of those two taxonomic groups, eDNA detected 100 taxa while Surber surveys captured an additional 61 taxa, indicating that eDNA was efficient




f. Covariate Analysis

| Species | ΔELPD No Covariate | ΔELPD BAM% | ΔELPD Mean Forest Stand Age | ΔELPD BAM% + Stand Age | Mean $\alpha_2$ (95% CrI) of Best Covariate Model |
|---|---|---|---|---|---|
| Coho Salmon | -2.7 | **0** | -4.5 | -2.7 | -4.218 (-7.661,-0.608) |
| Coastal Tailed Frog | -4.4 | **0** | -5.1 | -2 | -4.216 (-6.861,-1.509) |
| Signal Crayfish | -3.6 | **0** | * | * | 2.311 (0.513,4.398) |
| Red Legged Frog | **0** | -1.7 | * | * | NA |
| Rainbow Trout | **0** | -1.5 | * | * | NA |

* Markov chain Monte Carlo (MCMC) algorithms did not converge or too many divergent transitions after warmups because model did not explain the data.

**Fig. 6** eDNAJoint analysis comparing eDNA metabarcoding with traditional electrofishing. Survey units (number of eDNA field replicates or electrofishing reaches) required to detect (**a**) rainbow trout, (**b**) coastal tailed frog, (**c**) signal crayfish, (**d**) coho salmon, and (**e**) red legged frog as the species density (expected count/electrofishing sample) approaches 1 without accounting for covariates. Here, detecting the species refers to catching at least one individual in an electrofishing sample or producing at least one true positive eDNA detection with 0.9 probability. Panel (**f**) shows the impact of burn severity (BAM%), mean forest stand age, and both covariates combined on eDNA sensitivity relative to traditional electrofishing. Higher expected log pointwise predictive density (ELPD) corresponds to a more predictive model. $\alpha_2$ is the mean value of the covariate coefficient for site-level covariate regression that scales eDNA sample sensitivity relative to traditional sample sensitivity (95% credibility interval (CrI) in parenthesis). Positive regression coefficients correspond to an inverse relationship between the variable and probability of detection

at detecting other aquatic insect groups. Only 11% of the detections (30) shared the same identification as those from the traditional survey.

### Responses of at-risk species to wildfire

We obtained spatially extensive data on species with elevated conservation concern and designated as priority species for managed forests in the Pacific Northwest. These included federally or state-listed species, including species protected by the Oregon Forest Practice Act, species with the greatest conservation need in the Oregon Conservation Strategy or with a global or state status of G1 or S1 (critically imperiled), G2 or S2 (imperiled), or G3 or S3 (vulnerable) (NatureServe 2026, Oregon Department of Fish and Wildlife (ODFW), 2026, and Oregon Forest Resource Institute, 2017). We detected several at-risk species that occurred only in burned watersheds (Supplemental Fig. 5), including Chinook salmon (ESA Threatened), coho salmon (ESA Threatened), Pacific lamprey (OR S1), and western river lamprey (OR S4) that were detected in watersheds that were 78 to 100% burned. We also detected steelhead (ESA Threatened)/rainbow trout, red-legged frog (OR S3) and coastal-tailed frogs (Oregon S3) in burned and unburned watersheds.

## Discussion

Our spatially extensive examination of biodiversity using multiple methods provides evidence that pre-fire forest management of forest stand age did not interact with fire severity to influence aquatic species richness or measures of diversity during the first year after wildfire. Our combined approach of using eDNA metabarcoding and traditional analyses depicted diverse aquatic communities of vertebrate and invertebrate species across a range of prior disturbances including forest management and widespread wildfire. Our data showed subtle differences overall between burned and unburned streams suggesting that aquatic communities embedded in forested watersheds were resistant to substantial community changes associated with wildfire, but that also depended on sampling method and taxonomic grouping. These results provide limited support for the pyrodiversity-biodiversity hypothesis that fire can maintain biodiversity in fire-adapted landscapes and also suggest that native aquatic

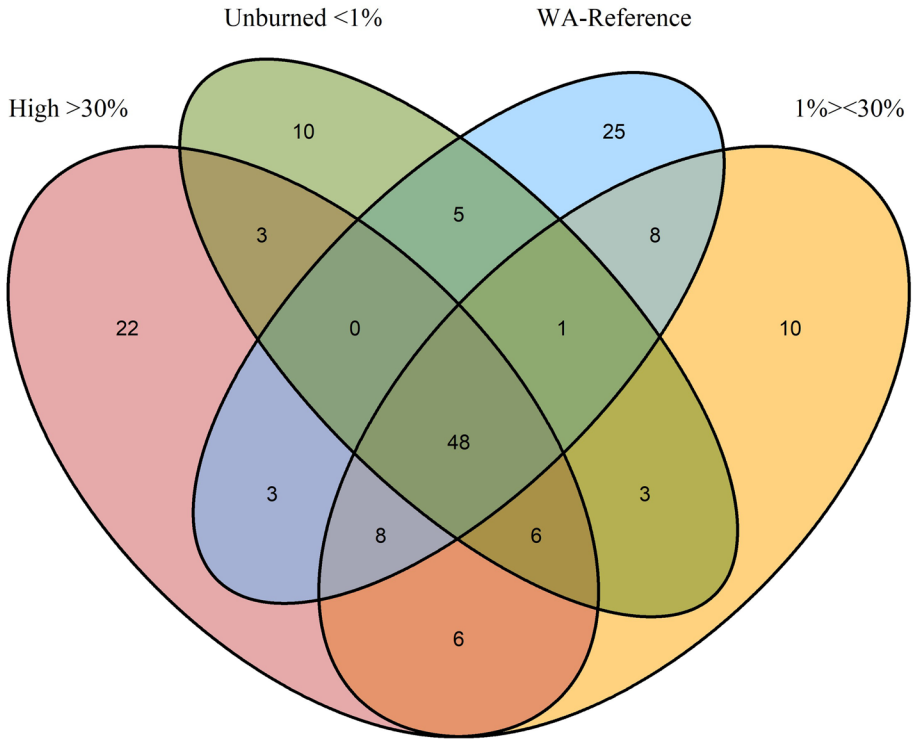



**Fig. 7** Shared and unique eDNA detections for Watershed Biodiversity across all site conditions grouped by unburned (< 1%) mean basal area mortality (BAM)%, low (1% > < 30%) BAM%, high (> 30%) BAM%, and Washington unburned reference sites

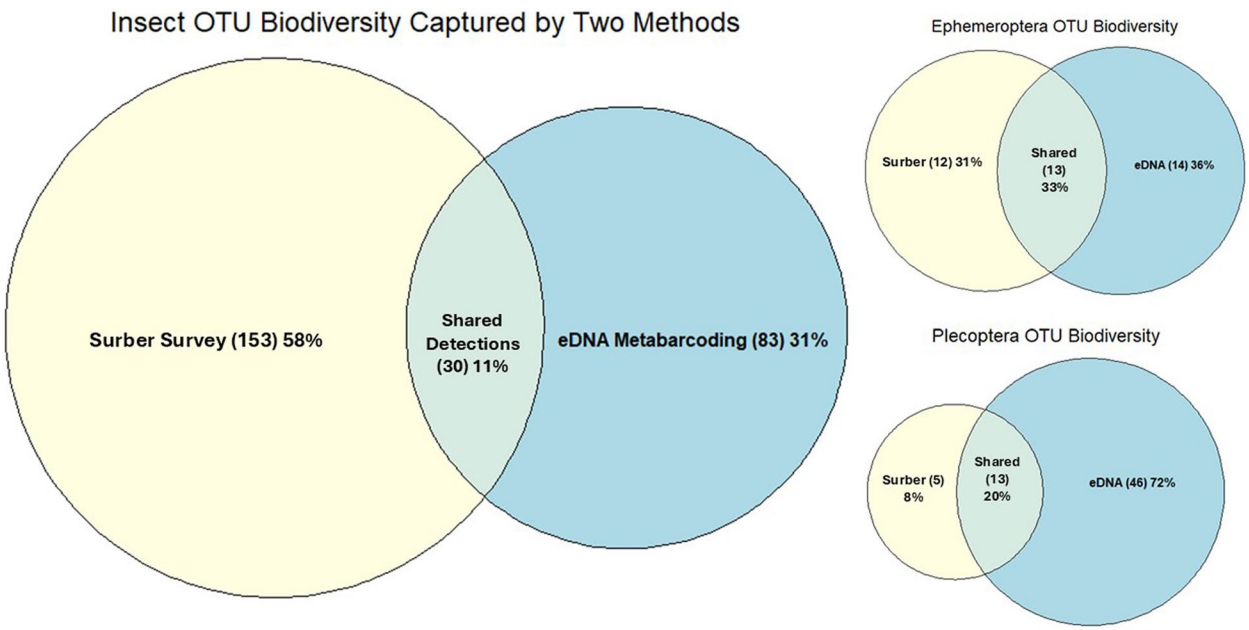



**Fig. 8** Aquatic insect OTU detections by eDNA metabarcoding and Surber survey as a comprehensive dataset and broken out by orders ephemeroptera and plecopteran

species in the Pacific Northwest may be fire adapted as has been observed elsewhere (Whitney et al. 2016).

Although we did not detect an interaction between fire severity and pre-fire forest stand age for biodiversity and richness metrics, we identified subtle shifts from both factors independent of one other. It is possible that with a larger number of samples we may have increased the possibility of detecting more statistically significant differences between our sample groups. Detections of aquatic insects using eDNA revealed greater species richness in sites from older forests (> 90 years) when compared to sites with a younger average stand age (Fig. 2b) independent of fire severity. Streams draining older mean forest stand ages in this study are also associated with higher elevation and different climate characteristics than streams draining younger mean stand ages in our study area (Coble et al. 2023) and macroinvertebrate diversity responses may also reflect these differences. However, our finding suggests that younger forests may not sustain as great a number of invertebrate species as older forests, which is a finding consistent with unburned streams in coastal Oregon where invertebrate diversity was also higher in older forests (Penaluna et al. 2026). We did not observe any changes in species richness as a function of fire severity for eDNA, Surber surveys, or electrofishing for any of our taxonomic groupings.

Prior research evaluating macroinvertebrate response to fire typically compared burned to unburned streams without evaluating a gradient in fire severity. Specific studies reported an increase in macroinvertebrate richness in Alberta 5years post-fire (Silins et al. 2014), no change in richness (Romme et al. 2011; Oliver et al. 2012; Robson et al. 2018; Martens et al. 2019–8 years after Alberta fire in Silins), or declines in richness (Roby and Azuma 1995; Rinne 1996; Minshall et al. 2001a; Minshall et al. 2001b; Earl and Blinn 2003; Minshall 2003; Verkaik et al. 2014) some indicating recovery to pre-fire conditions within a year or two of the fire disturbance (Minshall 2003). Other studies have observed no changes in species richness or diversity but documented changes in community composition (Oliver et al. 2012 and Minshall 2003). These prior studies used benthic sampling of macroinvertebrates, and different intervals of time post-fire that may complicate comparisons of response data. Our lack of observed response to fire with both eDNA and Surber sampling methods with aquatic insect data aligns with those studies that observed no changes or observed a recovery to pre-fire conditions 1 year post disturbance (our sampling interval after fire). We did observe some aquatic insect taxa only in burned or unburned locations with our eDNA data; for example, summit needlefly (*Paraleuctra vershina*), cataract forestfly (*Visoka cataractae*), and representatives from two stonefly families (*Capniidae* and *Chloroperlidae*) were only found in unburned sites while a *Malenka* species of Tiny Winter black stonefly, a species of green stonefly (*Sweltsa* sp.) and knobbed forestfly (*Soyedina producta*) were only found in burned locations. These observations suggest that there may be some shifts in community composition, and that those species found in burned areas may be more resilient in disturbed habitats than others. Martens et al. (2019) also found that *Capniidae* were strongly associated with reference catchments and not fire-damaged areas.

The Shannon's diversity index from electrofishing data showed a negative relationship with watershed burn severity (Fig. 3c), although the $R^2$ value indicates that only a portion of the variation is explained by this response and that stream biota were more unevenly distributed at higher burn severity. This suggests that some species were more abundant than others based on captures with electrofishing. Although we rarefied the eDNA data to allow for meaningful comparisons of diversity across samples with irregular read distribution, we failed to capture the decline in diversity unevenness with increasing fire severity observed by electrofishing. This may be a result of our eDNA targeting strategy with more primers targeting certain species of interest and masking relative abundance. For example, we had 5 primer sets specifically targeting trout and salmon, but only 2 that specifically targeted sculpins. At Norhorn Creek, traditional electrofishing captured 73 sculpin spp. individuals, 21 rainbow trout and 24 young-of-the-year trout. Relative abundance matrices should reflect more sculpin than trout; however, our eDNA detections at that location reveal a very strong signal for rainbow trout (457,532 average reads per replicate) and only moderate signal (64,672 average reads per replicate) for *Cottus* spp. (Fig. 5). Because our eDNA signal from the more abundant sculpins is less robust than the signal from the fewer trout, our Shannon index calculations for that location are skewed and do not reflect the correct relative abundance ratios to assess biodiversity with that matrix. Balancing primer targeting in eDNA metabarcoding studies may be a useful strategy to assess species evenness in future studies. In addition, neither method observed cutthroat trout at Norhorn, so the eDNA results would indicate that the young-of-the-year captured by electrofishing at this location were all rainbow trout.

Methodological agreement between eDNA and traditional electrofishing sampling has been well-validated in our study region (Hauck et al 2019; Penaluna et al 2021, 2023), and we also found good agreement in this study with eDNA identification of species, excluding aquatic insects. eDNA provided for improved understanding of sculpin lineages and identified young-of-the-year

salmonids to their genetic lineage (Penaluna et al 2023), both of which are difficult to identify to species in the field. eDNA also identified some fish species that we have not captured with electrofishing but are known to be present in these systems, such as minnows. Because electrofishing only captures species in the sampling reach while eDNA can be transported and detected downstream from a few meters in small streams to nearly 10 km in riverine systems depending on size and flow of the water being sampled (Deiner and Alternmatt, 2014, and Jane et al 2015) direct comparisons like ours of biodiversity across both methods are complicated. Our sampling approach focused on 4th order streams to best capture watershed-scale biodiversity with the assumption that some signal was transported from upstream.

While the eDNAJoint comparison was favorable for some species (with as few as 2 replicates needed for detection), it is unreasonable to consider that it would take 50 to 100 or more field replicates to detect other species, particularly when we did detect those species with only 8 field replicates. If we were to adjust mu to reflect the actual expected catch rate, we would likely see changes in the number of field replicates required. The eDNAJoint model did, however, reveal for us which species had lower detection rates and indicated how burn severity impacts our ability to detect some species. It is possible that altering our targeting strategy for the more difficult-to-detect species could increase the capture probabilities, and that those numbers could shift with more data fed into the model. With such a variable response to the burn severity covariate of BAM%, it is likely there are other fire-linked factors that are not fully captured by the environmental condition of that covariate. For example, fire can burn riparian vegetation that provides shade thereby increasing UV exposure of eDNA and water temperature degrading the DNA or there could be an increase in sediments post-fire making detections patchy. It is curious that the eDNAJoint model indicated that BAM% had both a positive effect on detecting a fish and a frog, no effect on a fish and a frog, and a negative effect on detecting a macroinvertebrate. This indicates that the response of detection to burn severity is at the species level, or that the results may be confounded by other untested environmental factors.

Relatively few studies have directly compared detection of aquatic insects with eDNA metabarcoding to traditional benthic sampling (Ji et al 2022, Fernandez et al. 2019, Takenaka 2024, meta-analysis by Poyntz-Wright et al. 2024), and this was our first opportunity to compare our 16S eDNA detections with traditional data from a Surber survey. Unfortunately, we failed to detect Trichoptera (caddisflies) and detected relatively few Diptera with eDNA. These failed captures indicate that our targeting strategy should be modified to include them, especially because caddisflies are an important water quality assessment tool within the EPT Index (Lenat 1988). Our success outside of those two taxonomic groups is not surprising as eDNA has been shown in some studies to detect more taxa than traditional methods when comparing eDNA collected from sediments (Ji et al. 2022). The relatively low overlap in common taxa with these two methods is likely due to a combination of the following factors: missed detections of caddisflies and flies by eDNA, eDNA detection of insects found upstream of the Surber survey, signals from persistent DNA shed by insects that have already metamorphosed and left the stream, collection of eDNA from water column while capturing macroinvertebrates from the benthos, and incongruencies between the species sampled and species sequences available in the reference database for the 16S region that we targeted. Many of our detections remained at higher taxonomic OTU levels, likely due to the lack of reference sequences for our amplicons in the reference database for this complex taxonomic group. Pinna (2021) and Takenaka (2024) discussed this issue for macroinvertebrates, and we encountered similar problems with mussel identifications (Hauck et al 2023). Species identification can also be confounded by traditional insect identification that might be lumping some species while genetic identifications are generating more refined resolution of species, such as we observed for young-of-the-year salmonids and sculpins. Reporting aquatic insect data as OTU richness helps to alleviate some of the problems encountered with molecular identification as this rapidly changing field develops. Aquatic insect surveys have been optimized in recent years by creating local databases that contain only expected taxa (Takenaka et al., 2024) and minimizing off target amplification with altered primer targeting strategies (Leese et al 2021) providing options for improvement in detections.

Inclusion of "all watershed" detections provided for a more complete understanding of the biodiversity in fire-affected locations and improved confidence in observed eDNA patterns that may have gone unnoticed otherwise. We did not, however, employ a traditional approach to validate observations for terrestrial taxa. Many of the taxa identified are known to occur in these watersheds and have been observed in other years (e.g., raccoon, American beaver, Dunn's salamander), but we do not have data from the date of sample collection to verify observations. eDNA metabarcoding was shown by Sales (2020) to provide a "terrestrial dividend" that captured a large portion of the expected species in the surrounding freshwater lotic ecosystem. We could improve our detections with various strategies. For example, we could collect water in a watering hole by a

game trail (Harper et al 2019) and deploy cameras to verify observations, or we might elevate the signal by increasing the number of metabarcoding primers on the array targeting species in the surrounding watershed. Altering our eDNA and traditional sampling strategy to better target terrestrial species may allow for a more comprehensive assessment of biodiversity in the surrounding watershed and provide additional context for disturbance studies.

## Conclusions

Our results represent the first eDNA metabarcoding approach to capture watershed-scale biodiversity to evaluate how aquatic ecosystems respond to wildfire. Because ecosystem responses may vary temporally, evaluating biodiversity at longer intervals post-fire may aid in evaluating additional disturbances from forest management, such as salvage, or delayed mortality of riparian and upland trees that influence habitat conditions, such as stream temperatures or allochthonous inputs (Dyer et al. 2025). The detection of at-risk species in burned watersheds (for example Chinook salmon and lamprey) suggests that suitable habitat occurred in the first year after wildfires, but additional sampling is necessary to ensure continued presence through changing conditions. As high-severity megafires increasingly affect forested landscapes, the need for species assessments becomes more urgent. Fire regimes affect the genetic patterns of fauna at an individual, population and meta-population scale by impacting both natural selection, survival and repopulation and dispersal (reviewed in Harris et al 2023). Use of eDNA metagenomics has been shown previously to capture the genetic diversity of pacific salmon (Weitemier et al 2021) providing proof of concept that eDNA metabarcoding may also provide valuable information to assess genetic diversity within surviving populations after fire.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42408-026-00521-4.

Supplementary Material 1.

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**Disclaimer**

The findings and conclusions in this publication are those of the authors and should not be construed to represent any official U.S. Government determination or policy. The use of trade or firm names is for reader information only and does not imply endorsement by the U.S. Government.

**Authors' contributions**

LLH developed the primers and metabarcoding targeting strategy and laboratory methods, conducted the bioinformatic sequence analysis, eDNAJoint analysis and Ranacapa analysis, generated figures and drafted the manuscript. JH, BEP and AC conceived the project idea, designed the sampling approach, secured funding, and drafted the manuscript. BEP designed and conducted the field work. AS conducted statistical analysis, generated figures, and drafted the manuscript.

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**Data availability**

These sequence data have been submitted to the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) and can be accessed by searching for BioProject Accession Number: PRJNA1302509.

## Declarations

**Competing interests**

The authors declare no competing interests.

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## Publisher's Note