Research Note

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Environmental DNA Sampling Identifies Different Nonnative and Native Salmonid Species Assemblages in Two Eastern Oregon Wilderness Areas

Abstract

Wilderness areas often provide critical habitat for native fishes. However, the remoteness of wilderness areas can limit fish monitoring efforts, such that changes in nonnative and native fish distributions may not be detected and management responses may be delayed. Environmental DNA (eDNA) sampling offers a non-invasive and efficient method to monitor aquatic species across remote waterways. We used eDNA sampling and species-specific qPCR analysis to assess distributions of nonnative brook trout (*Salvelinus fontinalis*) and native bull trout (*S. confluentus*) and Columbia River redband trout (*Oncorhynchus mykiss gairdneri*) across sections of Monument Rock and Strawberry Mountain Wilderness Areas in eastern Oregon. In Monument Rock, we did not detect brook trout or bull trout eDNA, supporting previous findings of brook trout non-invasion and bull trout extirpation. Redband trout eDNA was detected at 29 of 34 Monument Rock sites despite recent environmental disturbances. Environmental DNA from all species was detected in Strawberry Mountain. Brook trout eDNA was detected at 10 of 17 Strawberry Mountain sites, indicating upstream expansion of this nonnative species in Meadow Fork Big Creek. Bull trout eDNA was detected at seven sites and redband trout eDNA was detected at four sites, confirming native salmonid persistence despite decades of co-occurrence with brook trout. Brook trout eDNA concentrations were highest in samples closer to High Lake where the species was first introduced, while native salmonid eDNA concentrations were higher at locations further from the lake. This study provides baseline distributions for these species and a framework for using eDNA sampling to monitor fish distributions in remote areas.

Key Points

- We found that native salmonids are using habitat within Monument Rock and Strawberry Mountain Wilderness Areas.
- However, habitat degradation and expanding nonnative species may limit wilderness benefits to native salmonids in Monument Rock and Strawberry Mountain Wilderness Areas, respectively.
- This study provides a framework for using environmental DNA sampling to efficiently monitor fish distributions in remote waterways and to inform future management actions.

Keywords: brook trout, bull trout, headwater systems, monitoring, redband trout

Introduction

Federally designated wilderness areas are sections of land protected by the Wilderness Act of 1964 that are generally managed to preserve their "primeval" and "natural" character, while minimizing the effects of modern human activities (Wilderness Act of 1964, P.L. 88-577). As a result, wilderness areas often

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serve as a critical refuge for flora and fauna amidst widespread environmental change and anthropogenic disturbances (Cole and Landres 1996). Many wilderness areas contain higher elevation headwater habitat that maintains cold water temperatures (Bader 2000, Cordell et al. 2005), which will be essential for cold-water fishes under future climate scenarios (Isaak et al. 2015). Thus, wilderness areas are important for native fishes that require perennial access to cold water, including many species of native salmonids in the western USA (Hitt and Frissell 2000, Adams et al. 2001, High et al. 2008).

Because wilderness areas are managed to minimize human influence, many of these areas have been minimally impacted by resource extraction activities associated with native fish declines, such as logging, road construction, and mining (Baxter et al. 1998, Jelks et al. 2008, Sergeant et al. 2022). However, habitat in some wilderness areas may be degraded from uses that occurred prior to wilderness designation, or ongoing uses (e.g., livestock grazing) that are permitted under exceptions in the Wilderness Act of 1964 (Squillace 2014). Additionally, other disturbances, such as the expansion of nonnative aquatic species, can permeate wilderness areas through connected aquatic habitats. Nonnative salmonids, specifically, are one of the most widely introduced groups of aquatic invasive species in the western USA and detrimentally impact native salmonids through hybridization, predation, and resource competition (Hansen et al. 2019). Thus, the presence of nonnative salmonids threatens the "natural character" of wilderness areas (see Landres et al. 2015) and their long-term viability as a refuge for native aquatic species.

The remote nature of wilderness areas and management mandates that have promoted high quality habitat for native salmonids also increase logistical challenges for conducting standard fish monitoring activities in these areas. For instance, lack of roads and, with some limited exceptions, prohibited use of motorized equipment (e.g., motorized vehicles) may cause difficulties traveling to survey sites. Therefore, conventional sampling efforts (e.g., backpack electrofishing) in these areas may require additional time and resources relative to similar efforts in non-wilderness areas. These requirements may limit efforts to detect important changes in salmonid distributions (e.g., Pilliod et al. 2019, Isaak et al. 2022).

The collection and analysis of waterborne environmental DNA (eDNA) has unequivocally enhanced contemporary efforts to monitor distributions of aquatic species (Rees et al. 2014). Freshwater eDNA collection and analysis methods were first successfully demonstrated by Ficetola et al. (2008) for the detection of invasive American bullfrog (*Lithobates catesbeianus*) in wetlands. Subsequently, numerous studies have validated the efficacy of eDNA-based methods for delineating ranges of nonnative aquatic species (Carim et al. 2019), detecting fish species that persist at low density (Robinson et al. 2019), determining aquatic species' distribution boundaries with strong precision and accuracy (Penaluna et al. 2021), and efficiently sampling expansive and remote geographic areas with small personnel teams (McKelvey et al. 2016). Moreover, eDNA-based methods are non-invasive and are often more sensitive, cost-effective, and time-efficient for making inferences about target species' presence–absence than conventional sampling methods (Rees et al. 2014, Wilcox et al. 2016, Penaluna et al. 2021). Thus, eDNA-based methods offer great potential for monitoring changes in the distributions of nonnative and native salmonids in wilderness areas.

The brook trout (*Salvelinus fontinalis*) is the most widely distributed and abundant nonnative fish in the western USA (Schade and Bonar 2005) and detrimental effects of brook trout on native salmonids are well documented (Hansen et al. 2019). Bull trout (*S. confluentus*) and redband trout (*Oncorhynchus mykiss* ssp.) are among the native salmonids in the northwestern USA that presently persist across sections of their native ranges in sympatry with brook trout (Miller et al. 2014, Muhlfeld et al. 2015, Manning et al. 2022). The bull trout is a stenothermic cold-water specialist that has been listed as Threatened under the Endangered Species Act of 1973 (ESA; P.L. 93-205) since 1999 (USFWS 1999). This species has experienced widespread population declines due to habitat degradation and fragmentation (Rieman et al. 2007), warming water temperatures (Eby et al. 2014, Jones et al. 2014), and deleterious interactions with brook trout and other nonnative species (Gunckel et al. 2002, Manning et al. 2022). In habitats where

brook trout and bull trout are sympatric, the former often outcompetes the latter for resources (Gunckel et al. 2002, Voss et al. 2023), particularly in areas where water temperatures approach the upper thermal tolerance of bull trout (McMahon et al. 2007). In addition, brook trout hybridize with bull trout, leading to population declines from wasted reproductive effort and genomic extinction (Kanda et al. 2002). Although the redband trout is not currently ESA-listed, habitat degradation and deleterious interactions with nonnative species have reduced its distribution to approximately 42% of its historical range (Muhlfeld et al. 2015). Interactions specifically between brook trout and redband trout remain under-studied; however, research in southeast Oregon indicates that Great Basin redband trout (*O. mykiss newberrii*) persist at lower densities in habitats where brook trout are present (Miller et al. 2014).

Here, we use eDNA-based methods to assess the distributions of brook trout, bull trout, and Columbia River redband trout (*O. mykiss gairdneri*; hereafter "redband trout") in sections of two wilderness areas in eastern Oregon (Figure 1a). Substantial resources have been dedicated to native salmonid conservation and monitoring activities in the region; however, information detailing brook trout, bull trout, and redband trout distributions in these two wilderness area sections is outdated. Our objectives were to: 1) document the current distribution of nonnative and native salmonids, and 2) gain insights into the responses of native salmonids to wildfire and nonnative brook trout in these areas.

Methods

Study Sites and Background

Monument Rock Wilderness Area (hereafter "MRWA") is a 79 km^2 protected area in the southern Blue Mountains of eastern Oregon. The western slope of MRWA lies within the Malheur National Forest and encompasses the headwaters of the Little Malheur River (Figure 1b). The Little Malheur River is a tributary to the North Fork Malheur River and is part of the North Fork Malheur River Bull Trout Core Area (USFWS 2015b), the highest conservation priority bull trout core area in Oregon (Brignon et al. 2023).

Although brook trout have not been documented in the headwaters of the Little Malheur River basin in MRWA, local managers have expressed concern that brook trout may be present in or near MRWA and thus capable of expanding into designated critical bull trout habitat within this high-priority core area (Brandon Haslick, Burns Paiute Tribe Natural Resources Department, personal communication, 31 January 2023; Figure 1). Efforts to remove nonnative species from wilderness areas may be limited by geographic remoteness as well as opposing statutes within the Wilderness Act (Patterson 2015). Thus, early detection of brook trout is critical for evaluating intervention options before widespread expansion occurs. Bull trout were documented in the headwaters of the Little Malheur River in MRWA as late as 1967 (Goetz 1989) but were not encountered during surveys in 1989 (Buckman et al. 1992) and are presently thought to be extirpated from this area. Anecdotal sightings within the past 15 years suggest that bull trout or brook trout × bull trout hybrids may persist in or near MRWA (USFWS 2015c). While previous efforts have documented redband trout in MRWA (e.g., Bangs et al. 2008), their upper distribution limit in this area is unknown, with no assessments to document changes in the distribution of this species following recent disturbances. Specifically, the Rail Fire of 2016 burned the vast majority of MRWA, including high severity burn throughout much of the Little Malheur River riparian corridor within this wilderness area (Figure 2). Effects of this wildfire (e.g., debris flows, landslides, riparian vegetation alteration) may have detrimentally impacted redband trout, particularly in areas of high burn intensity (e.g., Rosenberger et al. 2015; Figure 2). In addition, ongoing cattle grazing in the Little Malheur basin has caused substantial impacts to instream and riparian habitat (Buckman et al. 1992, USFWS 2015c).

Strawberry Mountain Wilderness Area (hereafter "SMWA") is a 278 km^2 protected area in the Malheur National Forest in Grant County, Oregon. The southeastern slope of SMWA is drained by four tributaries to the Malheur River. These four tributaries form the headwaters of the Upper Malheur River Bull Trout Core Area (Figure 1a).

While there is little evidence of human activities degrading instream or riparian habitat in SMWA,

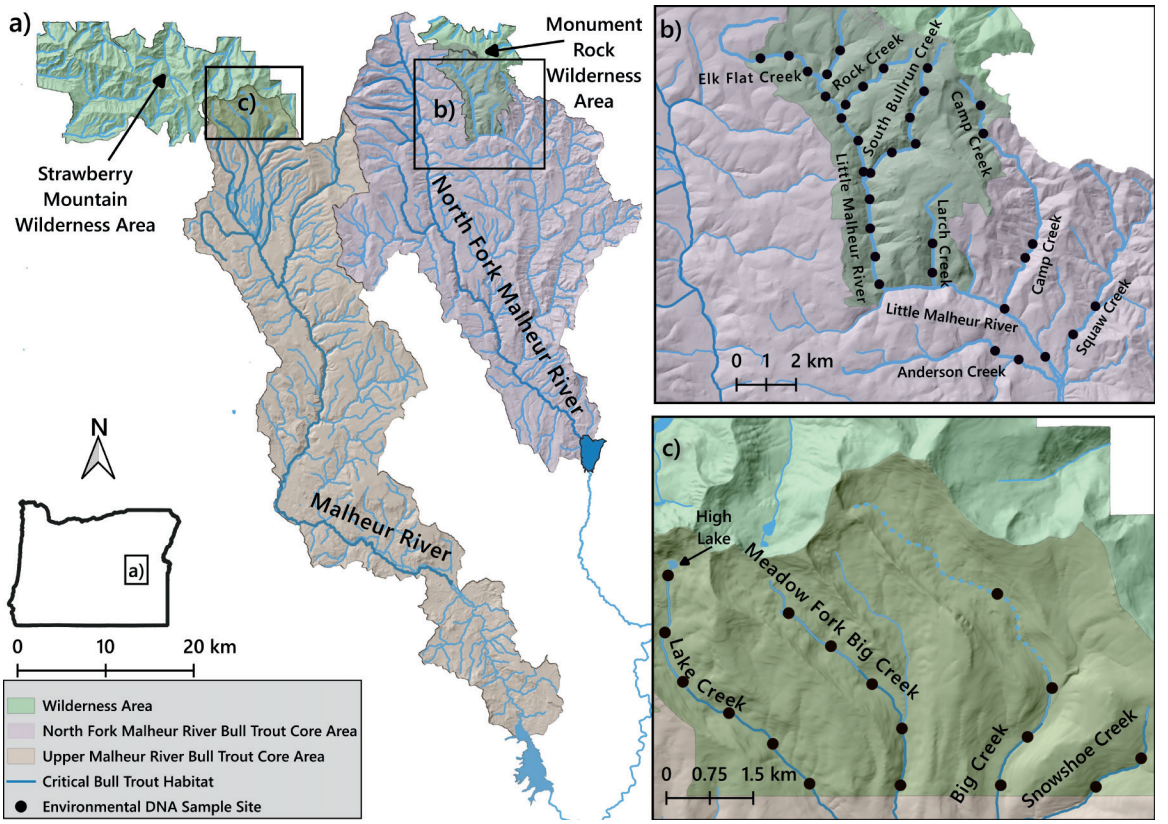

Figure 1. Map showing: a) overlapping area of the two wilderness areas and two bull trout core areas in eastern Oregon, b) environmental DNA (eDNA) sample collection sites (n = 34) distributed across the Little Malheur River and eight headwater tributaries in and around Monument Rock Wilderness Area, and c) eDNA sample collection sites (n = 17) distributed across four headwater tributaries to the Malheur River in Strawberry Mountain Wilderness Area.

nonnative brook trout were introduced to lakes in SMWA in the 1930s (Schwabe et al. 2004) and pose a threat to native salmonids in the area. High Lake (Figure 1c) was among the stocked lakes and is believed to be the primary source for recruitment and expansion of naturalized brook trout downstream into Lake Creek and other creeks within SMWA and the Upper Malheur River Bull Trout Core Area (DeHaan et al. 2010). Native bull trout and redband trout have been documented in the four Upper Malheur headwater tributaries in SMWA. Bull trout was previously reported as the most abundant species in the upper reaches of Big Creek, Meadow Fork Big Creek, and Snowshoe Creek, while redband trout was previously reported as the least abundant species in parts of this study area (Schwabe et al. 2004, DeHaan et al. 2010, Howell et al. 2018).

Environmental DNA Sample Collection

Environmental DNA sample sites were established at approximately 1-km intervals in perennial creeks and rivers where MRWA and SMWA overlap with the designated bull trout core areas (Figures 1b and 1c). We established 26 sample sites in MRWA and 17 sample sites in SMWA (Figure 1). Sampling in MRWA was completed between 9 and 15 July 2023, and sampling in SMWA was completed between 27 and 29 June 2024. Based on initial results from MRWA, we sampled eight additional locations downstream of MRWA on 30 June and 1 July 2024 (Figure 1b), for a total of 34 samples in the MRWA study area (Table S1). Samples downstream of MRWA were collected at locations suggested by local managers and were not established at 1-km intervals (Figure 1b). Sites in each

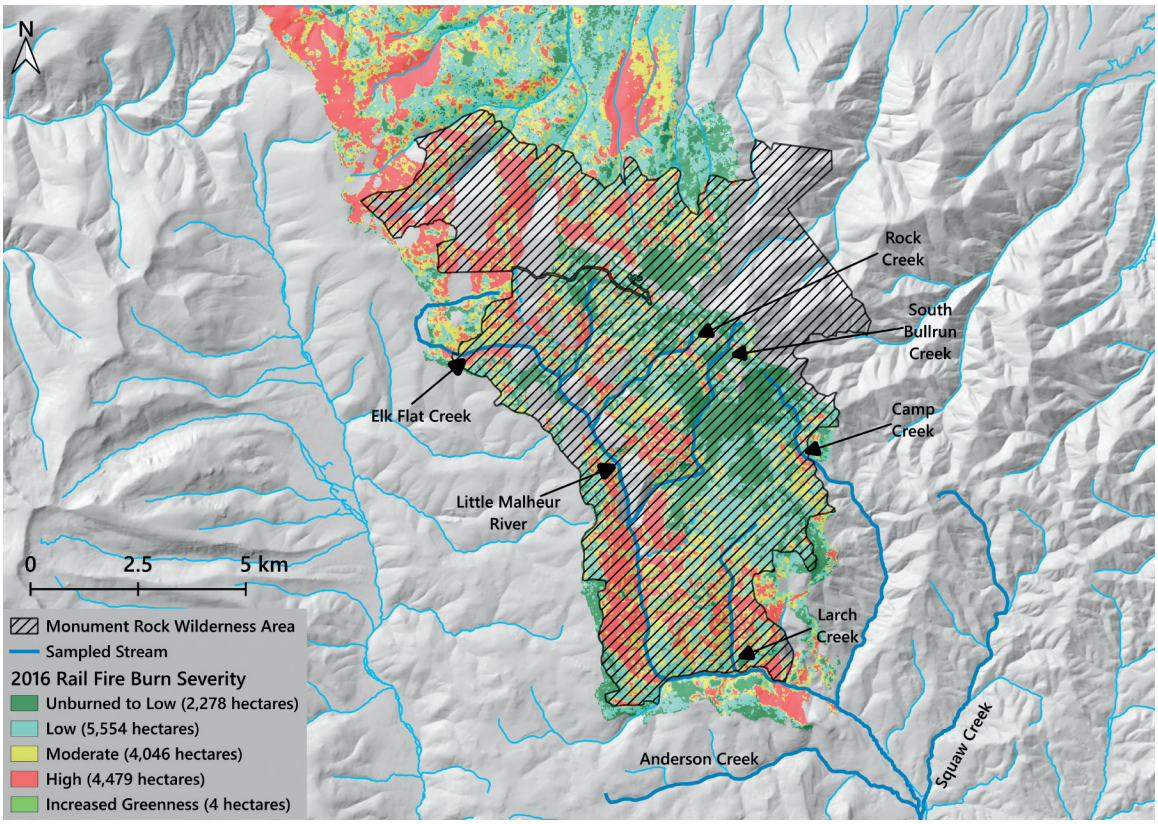

Figure 2. Map showing the 2016 Rail Fire burn severity, which included 17,729 hectares in and around Monument Rock Wilderness Area. Note that areas which did not receive a burn severity score due to masked reflectance imagery (1,367 hectares) are not included in the legend. Burn severity information retrieved from https://burnseverity.cr.usgs.gov/viewer/?product=MTBS.

waterway were generally numbered in a downstream to upstream orientation with Site 1 representing the furthest downstream sampling location.

Environmental DNA samples were collected following the protocol detailed in Carim et al. (2016b) using sampling supplies and materials provided by the U.S. Forest Service National Genomics Center for Wildlife and Fish Conservation (hereafter "NGC"; Missoula, MT). Briefly, a single sample was collected from the thalweg at each site using a peristaltic pump (Geotech) to filter up to 5 L of water through a 47 mm glass microfiber binder-free filter with 1.5 µm pores (Thermo Fisher Scientific). Samples were preserved in silica desiccant and stored at ambient temperature in a designated cooler during fieldwork and transport. At the end of each sampling trip, samples were transported to the State Fisheries Genomics Lab (Newport, OR) and stored at room temperature in a room separate from other potential sources of fish DNA (e.g., field equipment, tissue samples). Within 60 days of sample collection, samples were shipped to the NGC for laboratory analyses and long-term storage.

Environmental DNA Sample Analysis

Upon arrival at the NGC, sample metadata were recorded, and samples were stored at -20 °C until DNA extraction. DNA was extracted from half of each sample filter using DNeasy Blood and Tissue kits (Qiagen) and QIAshredder kits (Qiagen) following the protocol detailed in Carim et al. (2016a). The second half of each sample filter was retained and stored at -20 °C for future use.

Each sample was analyzed in triplicate for the presence and quantity of target species' DNA using quantitative PCR (qPCR) on a QuantStudio3 Real

Time PCR instrument (Thermo Fisher Scientific). Samples were analyzed using species-specific qPCR assays for brook trout, bull trout, and redband trout (Wilcox et al. 2013, Wilcox et al. 2015, Dysthe et al. 2018). A sample was considered positive for target species' DNA if amplification of target species' DNA occurred in at least one of the triplicate PCRs within 45 cycles (Wilcox et al. 2013). For all three assays, the limit of detection (LOD), defined as the lowest concentration at which 95% of positive samples are detected (see Bustin et al. 2009), was estimated at 10 copies per reaction (Wilcox et al. 2013, Wilcox et al. 2015, Dysthe et al. 2018), corresponding to approximately 100 copies per L in a 5 L sample. Samples with eDNA concentrations below the LOD may amplify inconsistently across PCR replicates and could therefore produce a false negative result.

All samples were analyzed alongside various controls to ensure the absence of contamination (negative control), assess for the presence of PCR inhibition (internal positive control [IPC]), and ensure that reactions were operating as expected (positive control). Each PCR plate included a negative control, which consisted of a triplicate reaction in which the DNA template was substituted with PCR-grade water. A TaqMan Exogeneous IPC Kit assay (Thermo Fisher Scientific) was added to each sample to test for PCR inhibition. A sample was considered inhibited if the mean cycle threshold (C_t) for the IPC across a sample's three replicates was delayed by > 1 cycle relative to the mean C_t of the negative control. To estimate eDNA concentration of the target species in a sample, all samples were analyzed alongside a five-level standard curve (6,250, 1,250, 250, 50, and 10 copies per 4 µl reaction) generated from a double-stranded gBlocks® Gene Fragment (Integrated DNA Technologies). Standard curves with efficiencies between 90–110% and goodness-of-fit (R^2) ≥ 0.99 were deemed acceptable for accuracy and precision (see Bustin et al. 2009). The standard curve also served as the positive control.

When samples are collected at consistent spatial intervals, the concentration of eDNA in a sample can be used to infer changes in population density of a target species across space (e.g., Wilcox et al. 2016, Baldigo et al. 2017, George et al. 2023). For samples in which eDNA from the target species was not detected, it is possible that one or more individuals were present upstream of the sampling location but were not present at high enough densities or in close enough proximity to result in eDNA detection (Jane et al. 2015, Wilcox et al. 2016). However, consistent negative results across consecutive sites can be used to infer species absence (see Carim et al. 2020). It is worth noting that the unique chemistry of each eDNA assay results in different detection efficiencies. Therefore, eDNA concentrations cannot be compared across assays to directly assess differences in densities among species detected at a given site. For example, eDNA concentrations of 150 copies per L for both bull trout and brook trout in a given sample should not be interpreted to mean that the two species are present at equal densities at the sampling location. However, changes in eDNA concentration across space for each species may be used to infer changes in relative density (e.g., as densities of one species increase, densities of another may decrease).

Results

Quality Assurance of Laboratory Results

No DNA amplification occurred in negative control wells. There was no evidence of PCR inhibition in any samples. Efficiency and goodness-of-fit (R^2) for standard curve analyses ranged from 93.3–102.2% and 0.990–0.997, respectively, satisfying recommended quality assurance guidelines for qPCR analysis (Bustin et al. 2009; Table S1).

Monument Rock Wilderness Area

No eDNA from brook trout or bull trout was detected in any samples collected in the MRWA study area (Table 1). Redband trout eDNA was detected in 29 of 34 samples, with concentrations ranging from 5–16,033 copies per L (Figure 3; Table S1). Redband trout eDNA was detected in all samples from Camp Creek, Elk Flat Creek, and the Little Malheur River (Table 1; Figure 3). Samples with the highest concentrations of redband trout eDNA were located within wilderness boundaries along the mainstem Little Malheur and just upstream of the confluence of major tributaries (Figure 3). At five sites, estimated redband trout eDNA concentrations were below the LOD (Table S1).

Table 1. Detections and non-detections of environmental DNA (eDNA) from three salmonid species in the Monument Rock Wilderness (MRWA) and Strawberry Mountain Wilderness (SMWA) study areas. *Includes sites downstream of wilderness boundary. **All sites established downstream of wilderness boundary.

Feature name	Study area	# of sample sites	Brook trout eDNA detected?	# of sites with brook trout eDNA detection	Bull trout eDNA detected?	# of sites with bull trout eDNA detection	Redband trout eDNA detected?	# of sites with redband trout eDNA detection
Anderson Creek**	MRWA	2	No	0	No	0	Yes	2
Camp Creek*	MRWA	5	No	0	No	0	Yes	5
Elk Flat Creek	MRWA	3	No	0	No	0	Yes	3
Larch Creek	MRWA	2	No	0	No	0	Yes	1
Little Malheur River*	MRWA	11	No	0	No	0	Yes	11
Rock Creek	MRWA	3	No	0	No	0	Yes	1
South Bullrun Creek	MRWA	6	No	0	No	0	Yes	4
Squaw Creek**	MRWA	2	No	0	No	0	Yes	2
Big Creek	SMWA	4	Yes	1	Yes	1	Yes	1
Lake Creek	SMWA	6	Yes	6	Yes	3	No	0
Meadow Fork Big Creek	SMWA	5	Yes	2	Yes	3	Yes	3
Snowshoe Creek	SMWA	2	Yes	1	No	0	No	0

Strawberry Mountain Wilderness Area

Brook trout eDNA was detected in 10 of 17 samples and eDNA concentrations ranged from 5–21,678 copies per L (Figure 4a). Based on eDNA detections, brook trout was the most widely distributed species in this area and was the only species detected in Snowshoe Creek and at the three highest elevation sites in Lake Creek (Table 1; Figure 4a). In addition, we detected brook trout eDNA approximately 3.5 km upstream from the previously known uppermost distribution of the species in Meadow Fork Big Creek (Howell et al. 2018). Brook trout eDNA concentrations were generally highest at sites closest to High Lake and throughout Lake Creek (Figure 4a). Brook trout eDNA concentrations were below the LOD at Big Creek Site 1 and both sites where positive detections occurred in Meadow Fork Big Creek (Figure 4a; Table S1). Bull trout eDNA was detected in seven of 17 samples and eDNA concentrations ranged from 22–388 copies per L. Estimated bull trout eDNA concentrations were below the LOD at the two uppermost sites in Lake Creek with positive detections (i.e., Lake Creek Sites 2 and 3; Figure 4b; Table S1). Bull trout eDNA concentrations were higher in Big Creek and Meadow Fork Big Creek and lower in Lake Creek (Figure 4b). Thus, brook trout and bull trout eDNA concentrations followed inverse, species-specific trends along the sampling gradient in this study area. Redband trout eDNA was detected in only four samples in Big Creek and Meadow Fork Big Creek, with eDNA concentrations ranging from 106–1,321 copies per L (Figure 4c). Notably, the second highest concentration of bull trout eDNA and highest concentration of redband trout eDNA occurred at Meadow Fork Big Creek Site 3, which is the only site where eDNA from both native species was detected and eDNA from brook trout was not detected.

Discussion

We found that native salmonids are still present in both wilderness areas despite habitat impairment and a recent wildfire in MRWA and the presence of nonnative brook trout in SMWA. In backpack electrofishing surveys conducted in 2007, Bangs et al. (2008) detected redband trout in the mainstem Little Malheur River and low elevation reaches of Camp Creek and Rock Creek in MRWA. Our study used eDNA analysis to assess salmonid distributions in MRWA in the first widespread aquatic assessment since the 2016 Rail Fire. We detected redband trout

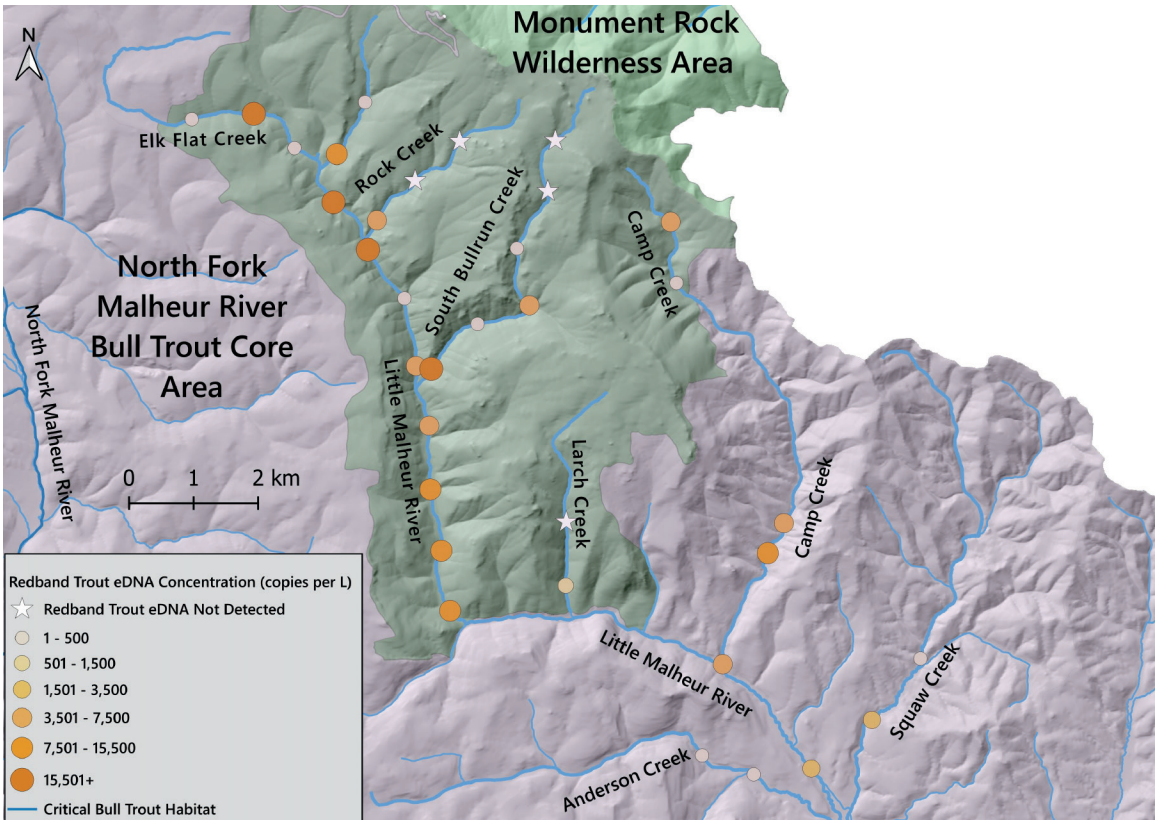

Figure 3. Map showing results of environmental DNA (eDNA) sampling in and around Monument Rock Wilderness Area. There were no detections of eDNA from brook trout nor bull trout in any samples. Redband trout eDNA detections ($n = 29$) are color- and size-scaled by estimated concentration.

eDNA at higher elevation reaches than those surveyed by Bangs et al. (2008), indicating that redband trout are present throughout the high elevation headwaters of the Little Malheur River basin in MRWA (Figure 3). Our results indicate that if the 2016 Rail Fire extirpated redband trout from MRWA, that extirpation was short-lived (e.g., Rieman et al. 1997). Furthermore, the highest redband trout eDNA concentrations in the Little Malheur basin occurred at sites within MRWA, suggesting that this wilderness may support high densities of redband trout compared to downstream sites (e.g., Searcy et al. 2022).

We did not detect brook trout eDNA in the MRWA study area, indicating that it remains free of this nonnative species. Additionally, eDNA from native bull trout was not detected in this basin, supporting previous findings that bull trout are extirpated from the MRWA study area. Bull Trout extirpation dates to at least the late 1980s and may be associated with a history of heavy livestock grazing in the area (Buckman et al. 1992). Habitat surveys conducted in 1989 noted that naturally low flows, along with impacts of ongoing, heavy livestock grazing likely made habitat in this area unsuitable for bull trout (Buckman et al. 1992). Restrictions on current grazing practices along with stream and riparian restoration in the Little Malheur basin have been identified as critical actions for improving bull trout habitat in the North Fork Malheur River Bull Trout Core Area (USFWS 2015c).

In contrast, habitat in SMWA has not been substantially altered by anthropogenic disturbances. In the early 2000s, stream condition surveys classified the upper sections of Big Creek, Lake Creek, and Meadow Fork Big Creek within the wilderness at nearly 100% of historic condition, while habitat

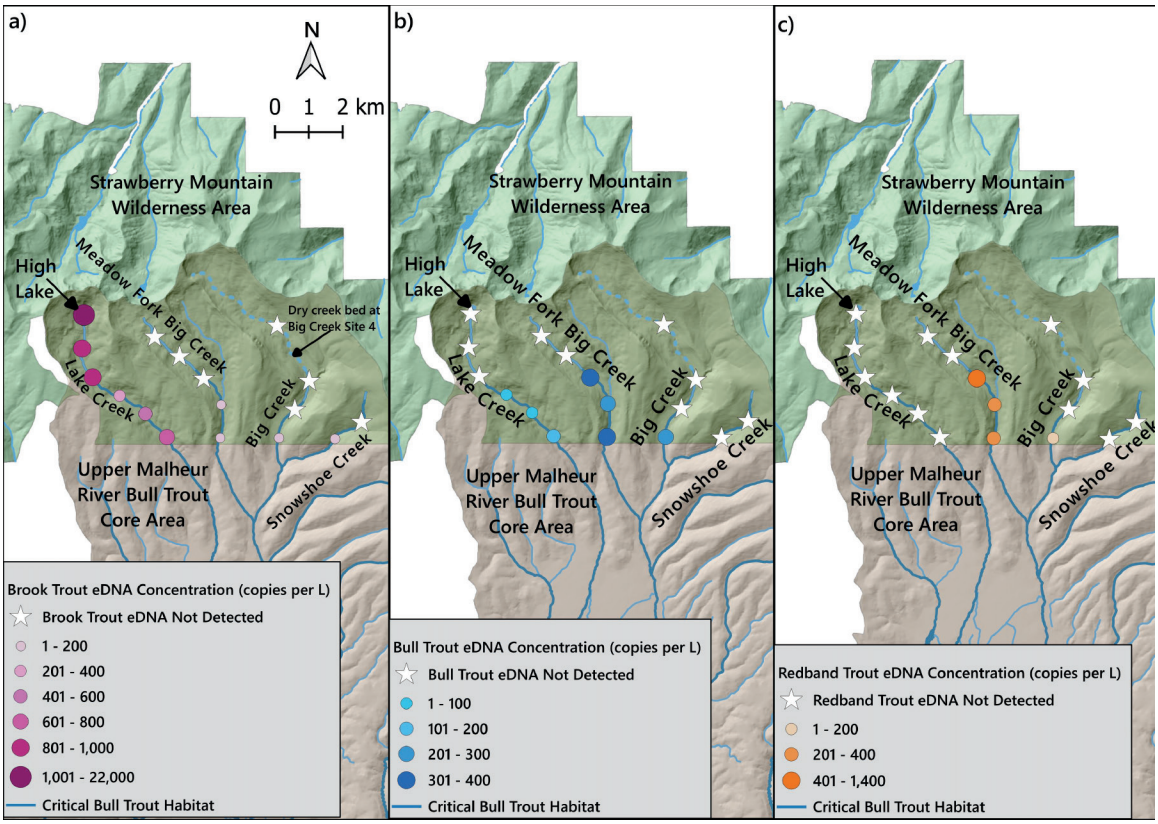

Figure 4. Map showing results of environmental DNA (eDNA) sampling in Strawberry Mountain Wilderness Area with species detections color- and size-scaled by eDNA concentration for: a) brook trout (n = 10), b) bull trout (n = 7), and c) redband trout (n = 4).

in the lower reaches of Big Creek and Lake Creek downstream of the wilderness was classified at 77% and 65% of historic condition, respectively (Malheur Watershed Council and Burns Paiute Tribe 2004, DeHaan et al. 2010). Instead, the widespread distribution of nonnative brook trout in this area is the largest threat to native salmonids, particularly bull trout (USFWS 2015a). For instance, competitive exclusion of bull trout and hybridization between the two species has been documented in the Upper Malheur River basin, including areas of SMWA (Gunckel et al. 2002, DeHaan et al. 2010) and across other areas of the northwestern USA (e.g., Kanda et al. 2002, Voss et al. 2023). In this study, estimated brook trout eDNA concentrations were highest in upper Lake Creek near High Lake and generally decreased at sites further away from the lake (Figure 4a). This trend in brook trout eDNA concentrations indicates that brook trout densities are higher near the original stocking location and lower in locations close to the current margins of expansion (Wilcox et al. 2016, Baldigo et al. 2017). Sites that had brook trout eDNA concentrations below the LOD were followed by multiple upstream samples in each tributary that were negative, further indicating lower brook trout densities at the margins of species distribution in Big Creek and Meadow Fork Big Creek. Bull trout eDNA concentrations were generally higher at sites in Big Creek and Meadow Fork Big Creek and lower in Lake Creek (Figure 4b). Estimated bull trout eDNA concentrations were below the LOD at Lake Creek Sites 2 and 3, while bull trout eDNA was not detected at the three uppermost sites in this tributary. This result suggests bull trout persist at lower densities at the upper margins of this species' distribution in Lake Creek. Redband trout eDNA was amplified at

sites in Big Creek and Meadow Fork Big Creek, but eDNA from this species was not detected in Lake Creek. Cumulatively, these findings suggest that the two native species persist at higher densities in areas where nonnative brook trout may be found at lower densities.

Moreover, we detected brook trout eDNA over 1 km upstream of the wilderness boundary in Meadow Fork Big Creek, indicating that upstream expansion has likely occurred since previous assessments (Schwabe et al. 2004, Howell et al. 2018). Although Meadow Fork Big Creek is predicted to provide suitably cold habitat for bull trout into the future (Isaak et al. 2015), the presence and expansion of brook trout presents a clear threat to their persistence. Specifically, the estimated probability of juvenile bull trout occurrence in Meadow Fork Big Creek for the years 2030–2059 is 47% in the absence of brook trout, but that probability decreases to 25% if brook trout are present (Isaak et al. 2015). Furthermore, the expanding co-occurrence of brook trout and bull trout warrants further investigations with genetic analyses to determine whether hybridization is becoming more prevalent since previous assessments (DeHaan et al. 2010).

There are several caveats to consider when interpreting our results. First, only one eDNA sample was collected at each site. Previous work has indicated that eDNA is exuded heterogeneously into the water column (Rourke et al. 2022), causing some variability in estimated eDNA concentrations collected in succession at the same site (Searcy et al. 2022). Accordingly, it is difficult to assess how accurately the estimated eDNA concentration from one sample per site represents a "true" mean concentration. In turn, this limits our ability to quantitatively link target species densities with estimated eDNA concentrations. Nevertheless, estimated eDNA concentrations from SMWA intuitively suggest that brook trout densities are higher near High Lake and lower at the margins of the current species distribution. Concurrently, eDNA concentrations from SMWA suggest that native salmonid densities are higher at sites further from High Lake. Second, due to remoteness of the study areas, we did not bring instruments to measure stream discharge at sample sites. In lotic systems, the probability of detecting eDNA from a target species and the concentration of estimated eDNA in a sample may be influenced by the quantity of water moving through a sample site (Levi et al. 2019, Yates et al. 2021). For instance, low discharge at a site may decrease the transport distance of eDNA particles from a source organism, thus reducing the probability of amplifying eDNA from organisms further upstream from the sample site (see Jane et al. 2015). Conversely, high discharge may dilute the number of eDNA particles moving past a site, thus reducing estimated eDNA concentrations and potentially confounding comparisons of estimated eDNA concentrations between sites with disparate discharge rates (Yates et al. 2021). Although similar discharge rates were qualitatively noted at sample sites in the SMWA study area, we acknowledge that varying discharge rates in the MRWA study area may have influenced estimated redband trout eDNA concentrations. Specifically, the Little Malheur River receives more tributary input as it flows through MRWA and downstream past the wilderness boundary (Figure 3). Consequently, higher discharge rates at downstream sites in the Little Malheur River may have diluted estimated redband trout eDNA concentrations.

Overall, our findings demonstrate that native salmonids have persisted in these wilderness habitats. However, our results do not suggest that their persistence in MRWA and SMWA is guaranteed, as threats remain from habitat degradation, increasing environmental disturbance, and the presence of nonnative brook trout. Although wilderness is generally managed to minimize human intervention, management actions to improve the natural character of wilderness and protect biodiversity are increasingly common, particularly when such actions counteract effects of anthropogenic activities (e.g., "trammeling"; Kelly and Landres 2023, Carim et al. 2026). For example, nonnative fish removals have occurred to promote native aquatic biodiversity in wilderness areas (DeMong 2001, Liss et al. 2002, Schnee et al. 2021). Similarly, interventions to maintain or improve habitat quality regularly occur in wilderness areas (Lieberman et al. 2018). Our results may inform targeted management interventions to improve the benefits of MRWA and SMWA to bull trout, redband trout, and other native species. These results also serve as a baseline for future monitoring efforts to assess the responses of native species to such efforts.

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Conflict of Interest

The authors declare no conflict of interest.

Animal Care and Use

No permits or Animal Care Committee compliance were necessary.

Data Availability Statement

Data generated from this project will be made publicly available through the Aquatic eDNAtlas database. All data supporting the findings of this work are available to download in Table S1 in the Supplementary Materials.

Supplementary Materials

Supplementary materials are hosted online by BioOne at https://doi.org/10.3955/046.099.0101.s1.

Author Contributions

All authors contributed to study conceptualization and funding acquisition. BW completed fieldwork, created figures, wrote the first draft, and edited subsequent drafts. KC and KO supervised BW and reviewed and edited drafts. All authors approved the final manuscript.

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