ARTICLE

Environmental DNA Sampling Informs Fish Eradication Efforts: Case Studies and Lessons Learned

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Abstract

Worldwide, freshwater ecosystems are threatened by invasive species, resulting in adverse effects on infrastructure, economy, recreation, and native aquatic communities. In stream settings, chemical piscicides can be an effective tool for eradicating invasive fishes. However, chemical treatments are expensive and time consuming, and they do not discriminate between invasive and native species in a system. Therefore, managers would ideally limit treatment to only the area occupied by the invasive species. Because traditional survey methods may not accurately detect individuals in low abundance (e.g., at the edge of their distribution, or following an eradication effort), chemical treatments may be applied more broadly and more often than is necessary to ensure complete coverage. Furthermore, inadequate post-treatment sampling can fail to detect survivors of a treatment. As a result, managers may erroneously conclude that eradication was successful, leaving the ecosystem vulnerable to reestablishment by the invader. More sensitive sampling tools should allow for more precise definition of the treatment area and more accurate evaluation of project success. This would reduce project costs and overall effects on native species. Here, we illustrate how environmental DNA (eDNA) sampling addressed these challenges through three case studies, each of which used eDNA sampling to inform the removal of Brook Trout *Salvelinus fontinalis* in small streams. We found that eDNA methods can be informative throughout all stages of eradication projects in stream settings. It can assist with delimiting the population

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prior to treatment, provide detailed location data on surviving target individuals, and serve as an efficient and relatively inexpensive monitoring tool to assess long-term treatment efficacy. When combined with traditional survey tools, such as electrofishing, eDNA sampling may help reduce the size and number of treatments that are necessary to reach project goals. This translates directly to increased efficacy of treatments, reduced labor and cost, and reduced adverse effects on the native community.

Freshwater ecosystems are among the most compromised in the world (Strayer and Dudgeon 2010; Williams et al. 2011), and invasive species are a primary cause (Carpenter et al. 2011; Huber and Geist 2017). The annual economic losses that are associated with aquatic invaders reach into the hundreds of billions of U.S. dollars (Lovell et al. 2006). Not surprisingly, managers often spend significant resources to suppress or eradicate invasive species. For example, treatments to remove Sea Lamprey *Petromyzon marinus* in the Saint Mary's River, Michigan, cost an estimated US$4.2 million per treatment (Lupi et al. 2003). In smaller water bodies, particularly small streams, efforts generally seek to eradicate the invasive species (e.g., Kulp and Moore 2000; Buktenica et al. 2013). This is often most effectively done through the application of a chemical piscicide (e.g., rotenone or antimycin; Meronek et al. 1996; McClay 2000). However, chemical treatments are nonselective and kill nontarget species, potentially disrupting the stream community (Hamilton et al. 2009; Vinson et al. 2010). Alternatively, repeated electrofishing can be successful at eradicating invasive fish populations in small streams with fewer effects on nontarget species. However, this method is exceptionally labor intensive and ineffective in streams with complex channels (Shepard et al. 2014). As a result, managers often employ a combination of these methods to meet the goals of an eradication project (e.g., Banish et al. 2019).

To reduce project costs and adverse biological effects on the native community, an eradication project ideally would treat the entire area that is occupied by the nonnative species, avoid areas where nonnative species are absent, and eliminate all target individuals in one treatment. In practice, however, eradication is usually an iterative process. Determining the extent of a nonnative species' distribution is difficult because densities—and therefore detection probabilities—are generally low at the margins of occupied habitat. This can lead to treatment of too much or too little of a stream network. Even when the treatment area encompasses the full distribution of the target species, multiple treatments are generally required due to a lack of concrete evidence confirming eradication (Britton et al. 2011). Repeated treatments could be more efficient if treatments could be limited to only the locations that support survivors from earlier efforts. However, given the low likelihood of detecting individuals at extremely low densities, the only way to ensure treatment success is to repeatedly treat the entire system. Therefore, methods that can increase the detection of fish at low densities will greatly improve the efficacy and efficiency of invasive species eradication projects in streams.

Environmental DNA (eDNA) sampling coupled with targeted PCR analyses (i.e., quantitative PCR or digital drop PCR) may be more effective than traditional methods for determining the presence and distribution of aquatic species, particularly when animals are rare (Jerde et al. 2011; Biggs et al. 2015; Sigsgaard et al. 2015; Wilcox et al. 2016). This is particularly true for small streams. For example, in streams with an average wetted width of 3 m, Wilcox et al. (2016) estimated that the probability of detecting a single 100-mm trout in a 100-m reach was 0.87 by using eDNA-based methods. In contrast, the average detection rates of stream-dwelling salmonids by using single-pass electrofishing range from 0.28 for Bull Trout *Salvelinus confluentus* to 0.58 for Brook Trout *Salvelinus fontinalis* (Peterson et al. 2004; Rosenberger and Dunham 2005; Letcher et al. 2015). Due to their sensitivity and specificity, eDNA methods are increasingly used to infer the extent of invasions (Dejean et al. 2012; Muha et al. 2017; Carim et al. 2019; Rubenson and Olden 2020) and assess eradication efforts in lentic systems (Dunker et al. 2016; Davison et al. 2017). Yet, there is no published information to date on how eDNA methods may be used to inform eradication efforts in lotic systems. Furthermore, no studies have provided guidance on how eDNA may be applied throughout different phases of an eradication project or provided tools to assist managers with the interpretation of eDNA-based detections when eDNA may be from nonliving sources (e.g., carcasses or stream sediment) rather than survivors of a treatment (but see Merkes et al. 2014).

Here we present three case studies in which rotenone treatments, electrofishing, and eDNA sampling were used in eradication projects targeting nonnative Brook Trout in northern Rocky Mountain streams. First, in Sage Creek, Montana, eDNA sampling was initially used to verify the success of a previous eradication effort. This led to the discovery of surviving Brook Trout and further eradication efforts. Later eDNA sampling provided information that allowed managers to reduce the size of subsequent treatments and provided evidence that the final eradication efforts were effective. Second, in Greenhorn Creek,

Montana, eDNA sampling was used to assess the efficacy of an eradication effort and to target follow-up sampling where detections were observed. Third, in Smalle Creek, Washington, eDNA sampling was used throughout all phases of the project, from defining the treatment area to assessing the effectiveness of each treatment. We use these case studies to illustrate the possibilities and challenges associated with using eDNA sampling to improve eradication projects in streams.

METHODS

Sample collection and laboratory procedures.— For all three case studies, eDNA samples were collected by pumping up to 5 L of stream water through a 1.5-μm glass microfiber filter (GE HealthCare) using a peristaltic pump (GeoTech Environmental Equipment, Inc.) following the protocol that was developed by Carim et al. (2016b). Each filter was placed in a plastic bag with silica desiccant and packaged in an envelope that was labeled with the sampling date, location name, and geographic coordinates. All of the samples were delivered to the National Genomics Center for Wildlife and Fish Conservation (Missoula, Montana) for analysis. Upon receipt, sample data were catalogued and the samples were stored at −20°C until they were analyzed. Sample DNA extractions were performed on half of each filter by using the Qiagen DNEasy Blood and Tissue Kit according to the protocol outlined by Carim et al. (2016a). The other half of the sample filter was retained and stored at −20°C. If more than one filter was used to collect the sample, DNA from all filters was combined during DNA extraction.

The samples were analyzed in triplicate by using quantitative PCR (qPCR) for the presence of Brook Trout DNA on a StepOnePlus qPCR instrument (Thermo Fisher Scientific) according to the methods that are outlined in Wilcox et al. (2013). Additionally, a subset of samples that was collected in Greenhorn Creek, Montana, were also analyzed for Westslope Cutthroat Trout *Oncorhynchus clarki lewisi* DNA according to the methods that are outlined by Wilcox et al. (2015). All of the reactions included an internal positive control to ensure that the reaction was effective and sensitive to the presence of Brook Trout DNA. A PCR reaction was considered inconclusive due to the presence of PCR inhibitors if the mean cycle threshold for the internal positive control across the triplicate reactions for a given sample was delayed >1 cycle compared to the no-template control. Without the use of internal positive controls, the presence of PCR inhibitors could mask the presence of target-species DNA, leading to false negative results (i.e., failure to detect a species that is present). If PCR inhibition was evident and the sample was positive for Brook Trout DNA, no further action was taken. If PCR inhibition was observed and Brook Trout

DNA was not detected, we treated the sample with an inhibitor removal kit (Zymo Research) and reanalyzed the sample in triplicate. The removal of inhibitors by using this method may result in some loss of DNA in a sample, although with elution volumes of 100–200 μL, the loss of DNA during inhibitor removal averages <10% (see http://www.zymoresearch.com for more details). To counter this effect, we extracted the second half of the sample filter and combined all of the extracted DNA from a given sample to obtain ~200 μL of extracted DNA. If a sample was still inhibited after treatment and negative for Brook Trout DNA, we analyzed the same total volume of DNA across four to six PCR replicates instead of three. Diluting the amount of DNA in a given PCR by increasing the ratio of water to DNA reduces the effects of inhibitors in PCR analysis (McKee et al. 2015). We increased the number of replicates after diluting the DNA to keep the total amount of DNA that was analyzed consistent. The results were considered inconclusive if PCR inhibition was still evident following inhibitor removal and sample dilution. In the Smalle Creek case study, the samples that were collected in June 2018 were from sites that had previously demonstrated high levels of inhibition. For these samples, inhibitor removal occurred prior to any assessment because conclusive eDNA results were needed as quickly as possible to determine whether another piscicide treatment was necessary that year.

When assessing an invasive species eradication project, false negative detections increase the chance of project failure. Because proximity to the target organism generally increases the probability of detecting that organism with eDNA (Pilliod et al. 2014; Balasingham et al. 2017), false negatives can be controlled by collecting samples at tight spatial intervals throughout the treatment area. Furthermore, we assumed presence based on minimum genetic evidence: a sample was considered positive if at least one reaction in the triplicate was positive for the target species' DNA after 45 PCR cycles.

False positive results can arise from several sources. Therefore precautions should also be taken to minimize these, as they could lead to unnecessary treatments and wasted resources. Legitimate but misleading positive detections can arise when live target individuals are not present but their DNA is as a consequence of decaying carcasses, transport from an outside source, or resuspension of eDNA from stream sediments (e.g., Merkes et al. 2014; Turner et al. 2015). These types of false positive detections may be common following an eradication effort because carcasses may be abundant following initial chemical treatments and DNA may be retained in sediment even if carcasses are removed. To minimize the occurrence of false positive detections from these sources, we collected eDNA samples at base flows no earlier than 9 months after a treatment. This allowed time for seasonal flooding

to flush the system and for any suspended sediment to settle. False positive detections can also result from DNA contamination that is introduced during field collection or laboratory analyses of eDNA samples. Negative control samples were assessed at multiple stages throughout the protocol to identify any contamination leading to false positive detections. The field equipment and sampling materials were assessed in the lab by testing one of every 100 sampling kits by filtering 0.5 L of bottled water as described above. To test for contamination during eDNA extraction, we processed an unused filter from every set of samples that was extracted (typically one extraction control per 23 samples). Finally, a negative control was included on each PCR plate to ensure that the PCR reagents and lab materials were not a source of contamination. As noted above, in the context of invasive species eradication, a false negative result may have greater consequences than a false positive result does. Therefore, we generally interpreted positive detections as real and proceeded with additional sampling to verify the results when necessary.

RESULTS

Case Studies

We present three case studies illustrating the use of eDNA methods to assess projects that were directed at eradicating Brook Trout from mountain streams. In all of the cases, the long-term management goal was to restore populations of native Cutthroat Trout *Oncorhynchus clarkii*. The application of eDNA sampling and the response to the results were unique to each project. Therefore, we present methods and results that are associated with each case study and follow with a more general discussion. The details of sample inhibition and qPCR analyses are presented in the Supplementary tables available in the online version of this article. For all of the projects, there was no evidence of contamination in any of the negative control samples.

Case 1: targeting retreatment, Sage Creek, Montana.— Sage Creek is a tributary of the Shoshone River with headwaters in the Pryor Mountains of Montana and Wyoming (Figure 1A). This 97,717-ha basin contains a mix of private lands and lands that are managed by the Crow Tribe, U.S. Forest Service, U.S. Bureau of Land Management, and the state of Montana. The upper forks and main stem are spring-fed while the lower sections are intermittently dewatered by several stock dams. Throughout the system, base flows range from 0.01–0.42 m^3/s in the main stem and <0.01–0.14 m^3/s in North Fork Sage Creek (M. P. Ruggles, unpublished data). Historically, the headwaters were occupied by Yellowstone Cutthroat Trout *O. c. bouvieri*, whereas the lower sections supported

a warmwater prairie fish assemblage. In 1933, a documented introduction of Brook Trout occurred in Piney Creek, a tributary to lower Sage Creek near Warren, Montana. Although there are no documented introductions of Brook Trout into Sage Creek, fish in Piney Creek may have migrated or been translocated into Sage Creek. In the main stem of Sage Creek, unknown subspecies of Cutthroat Trout *O. clarkii* sp. were periodically stocked from 1928 to 1942, as were Rainbow Trout *O. mykiss* from 1953 to 1983 (Montana Fish, Wildlife and Parks [MFWP], unpublished data). However, the spread of Brook Trout throughout the Sage Creek drainage is believed to have extirpated all *Oncorhynchus* spp. except for a relict population of Yellowstone Cutthroat Trout in Piney Creek (Ruggles, unpublished data).

The removal of Brook Trout in Sage Creek was a multiyear project that was led by MFWP in partnership with the Crow Tribe, U.S. Forest Service, U.S. Fish and Wildlife Service, and U.S. Bureau of Land Management. Rotenone treatments in 2010 and 2011 were focused on removing Brook Trout from roughly 50 km of stream in the Sage Creek basin. This included the lower 2.5 km of North Fork Sage Creek but not its upper 2.7 km, which was considered to be fishless based on electrofishing surveys. Through an agreement with local private land owners, MFWP stocked Yellowstone Cutthroat Trout in upper North Fork Sage Creek above the treatment area within a month of the initial 2010 treatment.

In 2010, managers considered an eradication project complete if no Brook Trout were observed during two consecutive years of electrofishing in the treated area. Based on this criterion, the treated area of Sage Creek was considered free of Brook Trout in July 2013. Later that month, while monitoring the previously stocked Yellowstone Cutthroat Trout in the untreated section of upper North Fork Sage Creek, managers discovered a reproducing population of Brook Trout. In September 2013, MFWP and partners treated the entire 5.2 km of North Fork Sage Creek with rotenone to remove the Brook Trout. Follow-up electrofishing surveys throughout all of the areas of Sage Creek that were treated between 2010 and 2014 were performed in July 2014 and 2015. No fish were observed during these electrofishing efforts.

In September 2015, we conducted coarse-scale eDNA sampling throughout Sage Creek to verify the apparent absence of Brook Trout (Figure 2A; Table S1 available in the Supplement in the online version of this article). This eDNA sampling was focused in areas with complex habitat (sections with undercut banks and beaver activity) that were more difficult to survey with traditional methods (Shepard et al. 2014). The sampling sites were selected based on flow rate, with sampling locations spaced at a 4-h flow interval (water at one sampling location would take
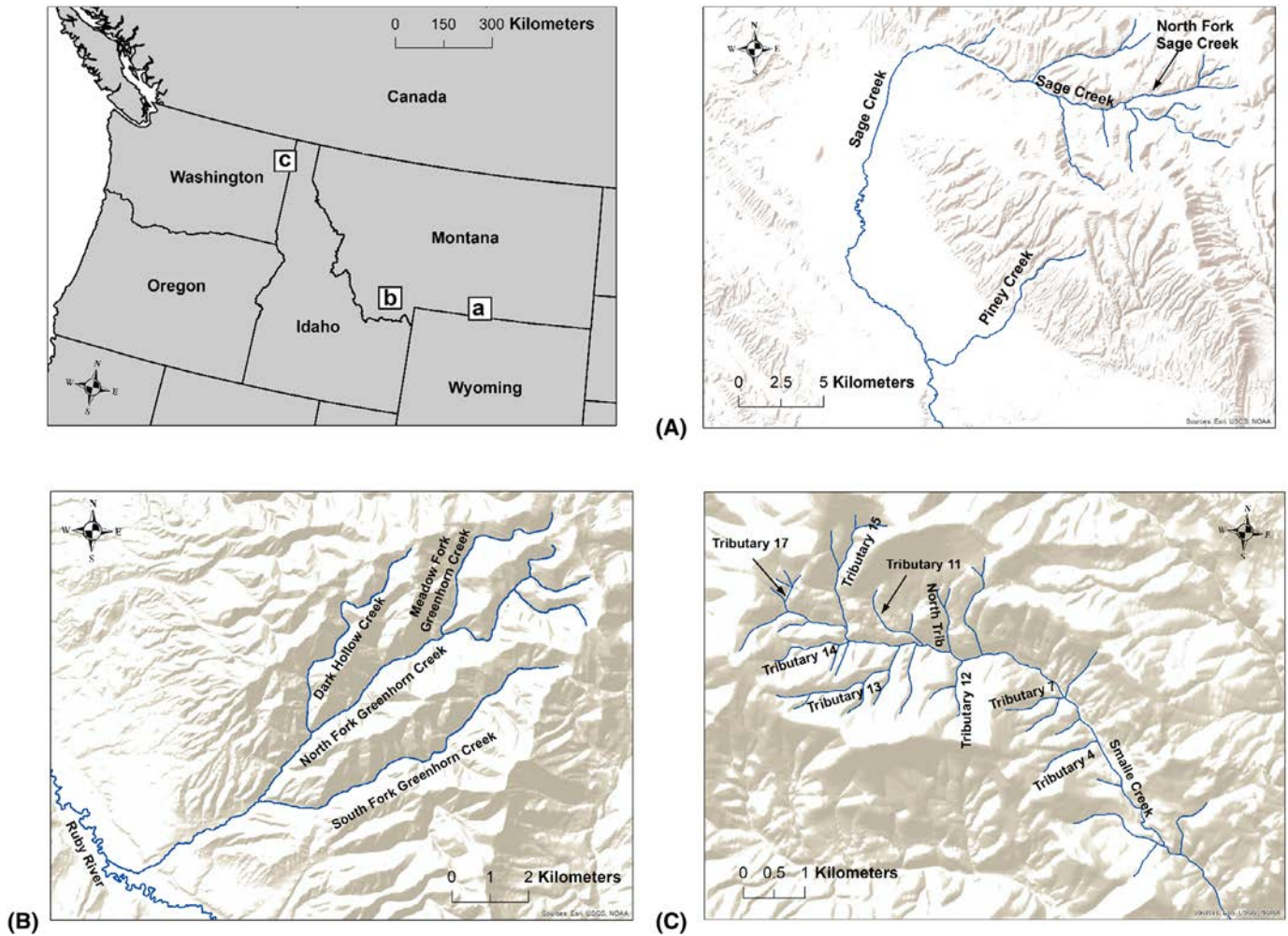
FIGURE 1. Locations of each case study using eDNA sampling to inform fish eradication efforts. [Color figure can be viewed at afsjournals.org]

4 h or less to reach the next downstream sampling location). Additional sampling locations were placed at 30-min flow intervals both up- and downstream of beaver complexes in North Fork Sage Creek to enhance the detection rates for Brook Trout in slow-moving water and complex habitat. Sixteen samples were collected from September 21 to 22, 2015, throughout Sage Creek; the distances between the sampling locations were 410–5,244 m (mean, 1,776 m; Figure 2A; Table S1). Brook Trout DNA was detected in two samples from North Fork Sage Creek, and electrofishing in December 2015 between sites 8 and 7 (approximately 1 km) resulted in the capture of a single Brook Trout. Conversely, Brook Trout DNA was not detected in the main-stem Sage Creek and Brook Trout were not observed through electrofishing for two consecutive years. As a result, we determined that further removal efforts were not necessary in the main stem and focused management activities on the removal of Brook Trout from North Fork Sage Creek.

To more precisely define the distribution of Brook Trout in North Fork Sage Creek, 20 additional eDNA samples were collected between May 4 and 9, 2016, in areas where the September 2015 eDNA survey indicated the presence of Brook Trout (Figure 2B; Table S1). The samples were collected at approximately 30-m flow intervals beginning at the bottom of the anticipated retreatment area and continuing 4.4 km upstream. Additional samples were added in areas with complex habitat and braided channels. Overall, the sampling locations were spaced 3–507 m apart (mean, 248 m). Brook Trout DNA was detected in 9 of these 20 samples, with one sample (at site 25) considered inconclusive due to persistent PCR inhibition. Positive eDNA detections identified roughly 1.8 km of habitat that was occupied by Brook Trout in North Fork Sage Creek. Later that month, follow-up electrofishing between sites 24 and 31 recovered four adults and many age-0 Brook Trout. The observation of live fish indicated that a rotenone treatment was necessary in
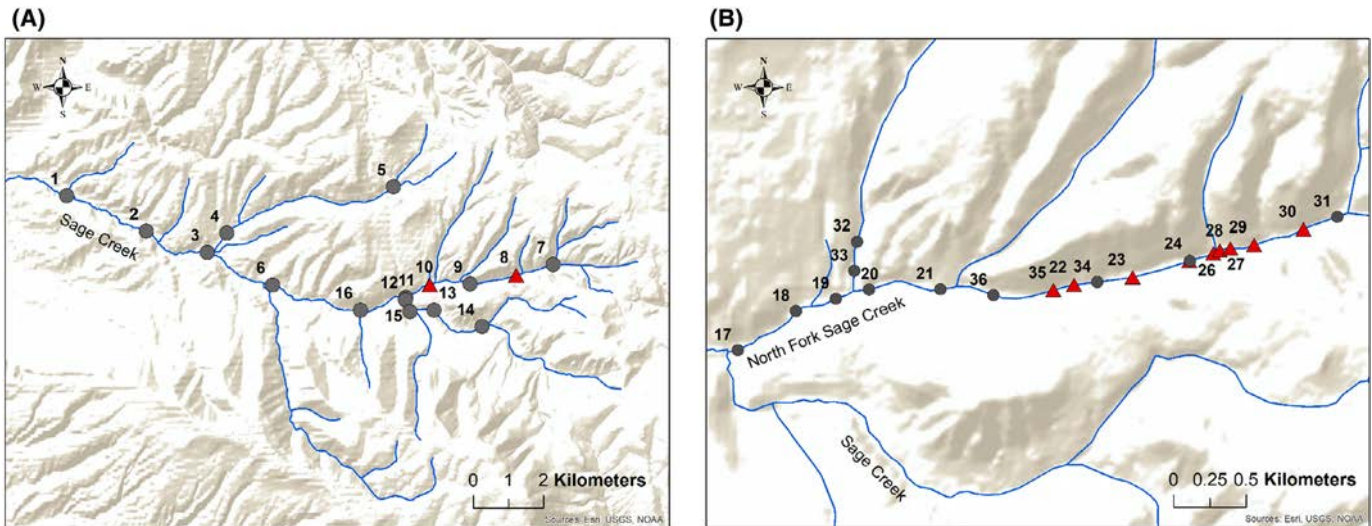
FIGURE 2. Environmental DNA sampling locations and results for detection of Brook Trout in Sage Creek, Montana, prior to rotenone treatment. **(A)** Coarse-scale sampling conducted in September 2015; **(B)** fine-scale sampling conducted in May 2016 to refine the treatment area. Red triangles represent sites with positive detections of Brook Trout eDNA; gray circles represents sites where Brook Trout eDNA was not detected. Labels refer to site ID in Table S1. [Color figure can be viewed at afsjournals.org]

North Fork Sage Creek. Because Brook Trout DNA was not detected in the samples downstream of site 36, the end of the treatment was moved up to site 36, reducing the anticipated retreatment area by approximately 1.9 km.

The second rotenone treatment in North Fork Sage Creek was applied on August 8, 2016, to 2.3 km of stream. On July 19, 2017, nearly a year posttreatment, five eDNA samples were collected at five locations throughout the North Fork Sage Creek treatment area (Figure 3; Table S1). Sampling at this time was limited by remaining project funds. Therefore, locations were strategically placed in areas with complex habitat and where live fish had been observed with electrofishing following the first treatment. The samples were spaced roughly 460–551 m apart (mean 499 m). This sampling effort was paired with electrofishing to further assess the effectiveness of the treatment. Brook Trout DNA was not detected in any of the samples, and no fish were observed during electrofishing. As a result, we concluded that no further eradication treatments were necessary on North Fork Sage Creek. The area received supplemental Yellowstone Cutthroat Trout stocks in 2018 to replace the fish that were lost during the treatment.

In the Sage Creek case study, the use of eDNA sampling provided assurance that 2010 removal efforts were successful at eradicating Brook Trout from the majority of the Sage Creek basin. In North Fork Sage Creek, eDNA sampling provided more precise information on Brook Trout distributions than electrofishing alone did. This information allowed managers to limit the extent of treatments only to areas where Brook Trout persisted and

to avoid retreating areas where Brook Trout had been removed. Finally, concurrent results between eDNA sampling and electrofishing at the end of the project provided evidence that no further removal efforts were needed. This allowed managers to reduce project assessment to 1 year of monitoring data and to reintroduce native Yellowstone Cutthroat Trout 1 year earlier. We estimate that these reductions in project scope and duration reduced the potential project costs by over 50%. Furthermore, adopting eDNA sampling at the onset of this project in 2010 could have led to an earlier discovery of Brook Trout in North Fork Sage Creek and further reduced the overall project duration and costs. However, laboratories that are capable of analyzing eDNA samples were not broadly available and an eDNA assay for Brook Trout (Wilcox et al. 2013) had not been developed at that time.

Case 2: posttreatment assessment, Greenhorn Creek, Montana.—Greenhorn Creek is a tributary of the Ruby River in south-central Montana (Figure 1B). In the project area, the stream flows through alpine meadows at higher elevations and conifer forest at lower elevations. The treatment area is almost entirely on federal public lands (1.5% private land, 66.4% Beaverhead National Forest, and 32.1% U.S. Bureau of Land Management). The upper basin comprises two main branches, the North and South Forks of Greenhorn Creek, which converge at a constructed barrier that is roughly 4.8 km upstream of the main-stem confluence with the Ruby River. Dark Hollow Creek enters the North Fork of Greenhorn Creek approximately 2 km upstream from the confluence of the north and south forks. Brook Trout and hybrids between
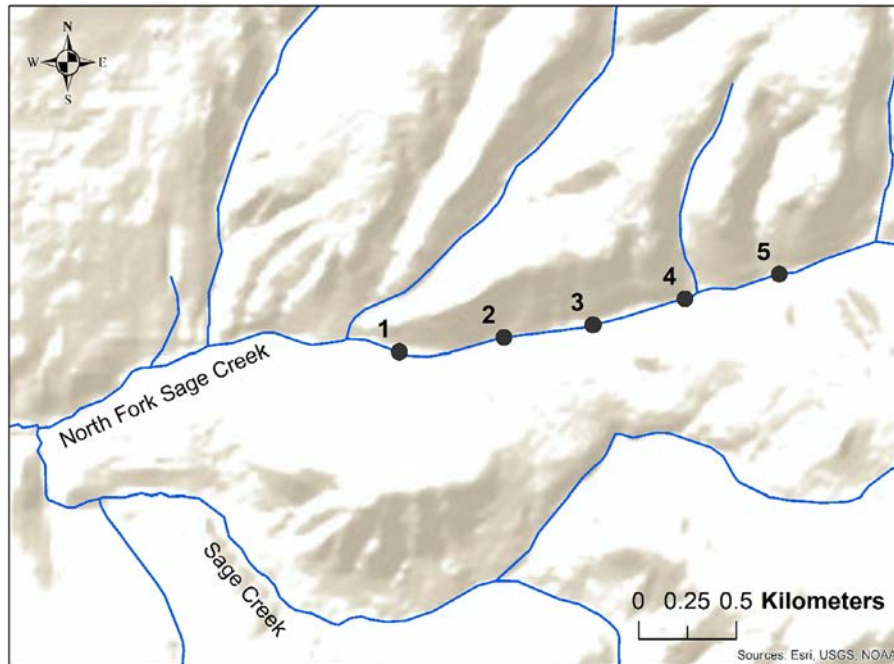
FIGURE 3. Locations of eDNA samples collected in North Fork Sage Creek, Montana in July 2017, 11 months after piscicide treatment. No eDNA of Brook Trout was detected in samples from any of the five sites. Labels refer to site ID in Table S1. [Color figure can be viewed at afsjournals.org]

Rainbow Trout and Westslope Cutthroat Trout were abundant immediately downstream of the barrier (Figure 2A). Stream flows downstream of the barrier range from $0.10\,m^3/s$ at base flow to $1.70\,m^3/s$ at bankfull flow (McCarthy et al. 2016).

Rotenone treatments to remove Brook Trout and hybrid Rainbow Trout × Westslope Cutthroat Trout were conducted by MWFP in 2013 and 2014 upstream of the barrier, except in Dark Hollow Creek, which hosted a population of genetically pure Westslope Cutthroat Trout. To determine whether additional treatments were necessary to remove nonnative trout, we assessed the entire basin upstream of the barrier in 2015 by using eDNA sampling. To gain confidence in the capability of eDNA to detect individual fish at fine scales, eDNA sampling was immediately followed with backpack electrofishing surveys. Between July 12 and 15, 2015, 122 eDNA samples were collected at 250-m intervals beginning just below the barrier and continuing upstream (Figure 4; Table S2). Jane et al. (2015) demonstrated 100% detection of Brook Trout in eDNA samples that were collected 240 m downstream of a small number of caged fish in headwater streams (at flows <2 L/s). Sampling at 250-m intervals in Greenhorn Creek allowed us to test this sampling interval more broadly. Single-pass backpack electrofishing was conducted throughout the project area within 12 h following eDNA sample collection. To reduce the risk of contamination from field equipment or gear, no crews or

equipment entered the system upstream of the eDNA sampling locations for at least 24 h prior to sample collection. All of the eDNA samples were analyzed for both Brook Trout and Westslope Cutthroat Trout except those that were collected in Dark Hollow Creek, which were only analyzed for Brook Trout.

Brook Trout DNA was detected in a positive control sample that was collected below the barrier (GBR) and at two locations within the treated area–sites NF31 and SF02 (Figure 4; Table S2). Westslope Cutthroat Trout DNA was also detected below the barrier, at three consecutive sites in Meadow Fork Greenhorn Creek (MF10–12) and one site in South Fork Greenhorn Creek (SF26). Electrofishing recovered a single Brook Trout roughly 160 m upstream of NF31 and a single Westslope Cutthroat Trout roughly 115 m upstream of MF12, corroborating the positive eDNA detections at both sites. There were no other Brook Trout or Westslope Cutthroat Trout observed via electrofishing in the basin, including in South Fork Greenhorn Creek.

In response to the positive detection of Westslope Cutthroat Trout at SF26, roughly 1,000 m of stream beginning at SF26 and extending up to SF30 was surveyed with electrofishing between August 10 and 13, 2015; no fish were observed during this effort. Because Westslope Cutthroat Trout were native to the system, no further effort was devoted to investigating this detection. In response to the detection of Brook Trout DNA at SF02, six additional
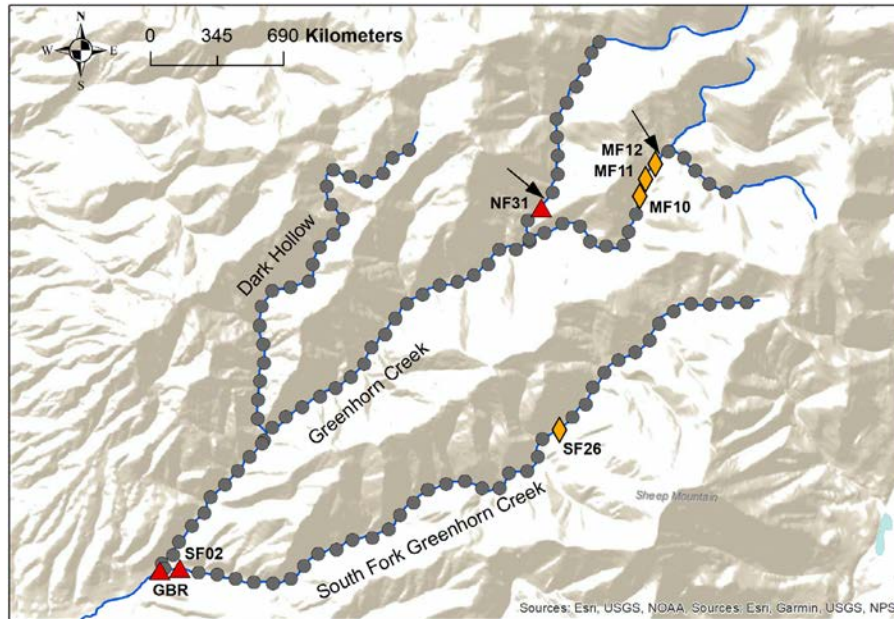
FIGURE 4. Results of eDNA sampling for detection of Brook Trout and Westslope Cutthroat Trout DNA in Greenhorn Creek, Montana. Site labels are only shown for sites with positive eDNA detections and refer to map ID in Table S2. Red triangles represent positive detections of Brook Trout eDNA; yellow diamonds represent positive detections of Westslope Cutthroat Trout eDNA; gray circles represent samples that were negative for DNA of both species. (Note that samples from Dark Hollow were only tested for Brook Trout eDNA.) The site labeled GBR is located immediately downstream of the barrier to fish passage. Arrows point to locations where live fish were recovered within 24 hours of eDNA sampling. [Color figure can be viewed at afsjournals.org]

eDNA samples were collected at SF03 and SF04 on September 13, 2015 (Table S2), followed by intensive electrofishing in the lower 1,000 m of South Fork Greenhorn Creek, spanning sites SF01 through SF05. Additionally, gill nets were set in a backwater reach immediately upstream of the barrier, and eDNA samples were collected from the spillover of the dam (before it contacted the stream below where Brook Trout were present). All of the subsequent eDNA samples were negative for Brook Trout DNA, and no fish were recovered with electrofishing or gill nets. With no further observations of live fish or eDNA-based detections of Brook Trout, we concluded that no further removal efforts were necessary. To help repopulate the Greenhorn Creek basin, MFWP biologists translocated nearly 700 Westslope Cutthroat Trout between 2016 and 2018 from six nearby populations.

In the Greenhorn Creek case study, the results of eDNA sampling at 126 of 128 total sites was concordant with electrofishing-based observations, supporting the absence of the target species across broad portions of the basin as well as the local presence of survivors of the chemical treatment. However, the sources of the positive detections at SF02 and SF26 remain unknown. No DNA of either target species was detected in any negative controls (including controls for equipment, sampling materials, extraction, and qPCR analysis), indicating that contamination from these sources was unlikely. It is possible that detections may

have been the result of contamination that occurred during the act of sampling itself such as DNA from waders or equipment that came into direct contact with the water that was being sampled. However, the lack of Brook Trout and Westslope Cutthroat Trout DNA in over 120 other samples that were collected in the basin suggests that this type of contamination was unlikely.

Alternatively, the positive detection of Brook Trout DNA at SF02 may have resulted from DNA that entered the system from a nonliving or indirect source other than the sampling process. For example, eDNA may have been transported on the surface of watercrafts or individuals that were moving from areas where the target species was present at high densities (see Jerde et al. 2013). Additionally, eDNA can persist in sediment and the feces of predators for weeks to months (Merkes et al. 2014). Prior to eradication, lower South Fork Greenhorn Creek hosted the highest densities of Brook Trout in the system and high densities of Brook Trout were present below the barrier, less than 250 m away (overland). While the source of Brook Trout eDNA in the initial sample from SF02 remains unknown, follow up eDNA and electrofishing efforts spanning this section provided sufficient evidence to conclude that no further sampling or eradication efforts were necessary.

The detection of Westslope Cutthroat Trout at SF26 is more puzzling. It is possible that a Westslope Cutthroat

Trout swam the 9 river kilometers from Dark Hollow Creek to that location and was only detected by eDNA sampling. However, the upper reaches of South Fork Greenhorn Creek are small and confined (<1 m wetted width at the time of sampling) with little habitat complexity; therefore, it is unlikely that a fish would escape electrofishing. Because the distance to Dark Hollow is more than 4 km overland, it is unlikely that DNA was transported to this location by another animal. Thus, an alternative explanation is that this detection represents an isolated, low-level contamination event that could not be traced to a specific point in the process of collecting or analyzing the eDNA samples.

Case 3: eDNA assessment throughout all project phases, Smalle Creek, Washington.— Smalle Creek is a tributary to Calispell Creek (a tributary to the Pend Oreille River) in northeastern Washington (Figure 1C). Two 10–20-m bedrock waterfalls that are located at river kilometer 9.9 (Hansen Falls) and 11.7 (Smalle Creek Falls) are barriers to upstream fish movement and represent the lower extent of the project area. Above Smalle Creek Falls, the main stem extends upstream 9.1 km and is fed by 17 tributaries, some of which occasionally go dry in summer months. The Smalle Creek project area is approximately 70% publicly owned (Colville National Forest) and 30% privately owned by a local timber company. Legacy effects from historical timber harvest were present throughout the area including abandoned logging roads, instream corduroy roads, and stands of timber in various states of regeneration. The base flows that were measured at Smalle Creek Falls between 2015 and 2018 were 0.03–0.08 m^3/s. Electrofishing surveys conducted in 2014 indicated that 8.3 km of main-stem Smalle Creek and portions of eight tributaries (4.8 km total) were inhabited by Brook Trout; no other fish species were observed. The remaining nine tributaries were presumed fishless based on a combination of electrofishing, presence of impassable barriers, and ephemeral stream flows.

Beginning in 2015, the Kalispel Tribe of Indians and the Washington Department of Fish and Wildlife began a multiyear rotenone treatment effort to remove Brook Trout from Smalle Creek above Smalle Creek Falls. The first treatment was originally planned for early September 2015, but regional wildfires delayed the treatment until October 14, 2015, after Brook Trout had spawned. Salmonid eggs are less susceptible to rotenone treatment than are older life stages (Marking and Bills 1976). Therefore, some proportion of the eggs that were deposited prior to the piscicide treatment was expected to survive and hatch the following spring. Managers proceeded knowing that at least one additional treatment would be necessary to eradicate fish spawned in 2015.

The distribution of Brook Trout prior to treatment was initially delineated by combining information from electrofishing surveys and stream characteristics that are generally considered unsuitable for Brook Trout (e.g., low flow, high gradient, etc.). The location of the highest elevation drip station in each tributary and the main stem was placed at least 150 m upstream of the last observed fish and typically above a habitat barrier that would impede upstream movement (such as a steep cascade or sharp increase in gradient). Prior to the first rotenone treatment, eDNA samples were collected to further inform whether the treatment area encompassed the distribution of Brook Trout and that Brook Trout were absent from the tributaries that were presumed to be fishless. Ten eDNA samples were collected between October 7 and 8, 2015, at the highest-elevation treatment station in the main stem and each fish-bearing tributary. Four samples were also collected just upstream of the confluence of the tributaries that were presumed to be fishless, which were still water-bearing at the time of sample collection (Figure 5; Table S3).

All of the samples that were collected at the highest-elevation treatment station in the main stem and tributaries were negative for Brook Trout DNA. This further increased confidence that the treatment area encompassed the distribution of Brook Trout in the Smalle Creek drainage. Of the four samples that were collected in the tributaries that were presumed to be fishless, only site (NT-1) in north trib was positive for Brook Trout DNA. North trib is a steep stream with a series of cascades, which includes a 3-m vertical drop that is roughly 100 m upstream of its confluence with Smalle Creek. No Brook Trout were observed with electrofishing surveys upstream of the cascades prior to treatment, and the lower 100 m was thought to be fishless due to low flows and the poor-quality habitat. To avoid any last minute changes during the 2015 treatment, and with the knowledge that a 2016 treatment would be necessary, a treatment station was not added to north trib in 2015.

Over the course of the entire project, all of the observed carcasses were removed from the stream immediately following each treatment by the Kalispel Tribe of Indians and Washington Department of Fish and Wildlife. This facilitated the removal of nonliving sources of DNA that could otherwise confound the interpretation of the eDNA sampling results in subsequent sampling efforts. Although an estimated 3,500 Brook Trout were removed from the project area during the initial treatment (N. J. Bean and J. M. Connor, unpublished data), insufficient rotenone concentrations and cold stream temperatures reduced the mortality rate of Brook Trout. Live adults were observed in main-stem Smalle Creek between treatment stations MS9 and MS8 and between treatment stations MS7 and MS6. Because live Brook Trout were easily observed post-treatment in the upper elevations of the treatment area and a large number of mature adults spawned in 2015
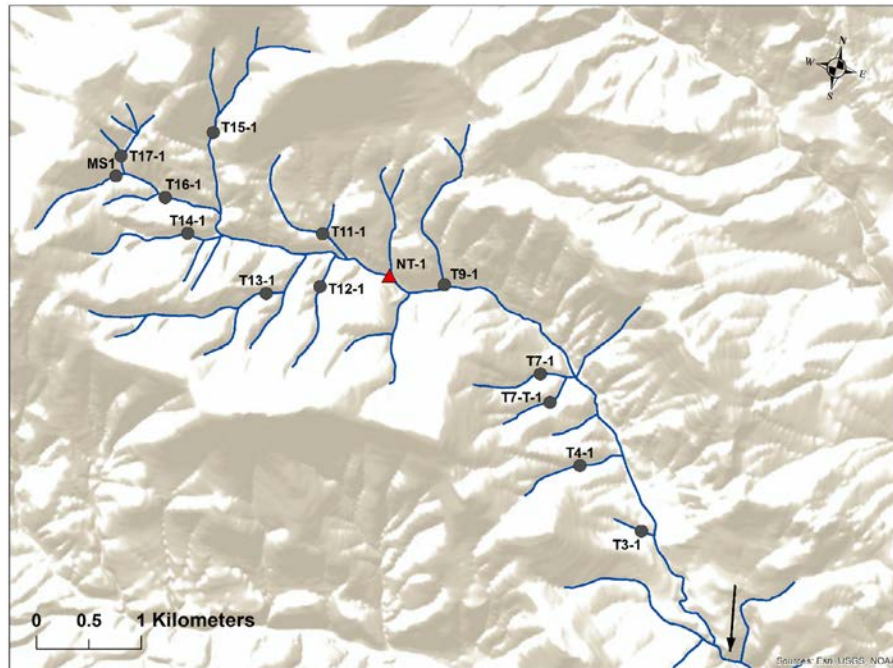
FIGURE 5. Results of eDNA sampling for detection of Brook Trout DNA in Smalle Creek, Washington prior to the first chemical treatment in October 2015. The arrow denotes the location of Hansen Falls, a natural barrier to upstream fish passage and lower end of the project area. Red triangles represent sites with positive detections of Brook Trout eDNA; gray circles represents sites where Brook Trout eDNA was not detected. Site labels refer to map ID in Table S2. [Color figure can be viewed at afsjournals.org]

prior to treatment, eDNA sampling was unnecessary to inform or justify subsequent treatment.

The second rotenone treatment occurred on September 14, 2016, prior to Brook Trout spawning. The 2016 treatment area included the same area as in 2015 as well as an additional treatment station 500 m upstream of the confluence with north trib. A total of 2,666 carcasses were removed from the stream following treatment, roughly 95% of which were age-0 fish ≤ 70 mm total length that had survived the 2015 treatment as eggs (Bean and Connor, unpublished data). To minimize the detections of Brook Trout DNA resulting from unrecovered carcasses or DNA that was sequestered in sediment (e.g., Jerde et al. 2016; Shogren et al. 2017), eDNA sampling did not resume until after the 2017 spring freshet.

To assess the distribution of Brook Trout following the 2016 treatment, we collected eDNA samples between June 5 and 8, 2017, at 39 main-stem and 18 tributary locations throughout the Smalle Creek drainage (Figure 6; Table S4). In Greenhorn Creek, eDNA of live fish was detected in samples that were collected 200–750 m from a live fish. With this in mind, we attempted to keep the distance between samples to 250–300 m. In the main stem, we collected samples at all of the rotenone stations and added intermediate sites as necessary to reduce the distance between sampling locations. In the fish-bearing tributaries, samples were collected 130–285 m downstream of the highest-elevation treatment station as well as just upstream of the confluence with the main stem, with the exception of tributary 17 and north trib. Tributary 17 was assessed with the sample that was collected in the main stem at MS2, located immediately downstream of the confluence of tributary 17, and 170 m downstream from the tributary 17 treatment station. North trib was assessed with a sample that was collected 40 m upstream of the confluence. Overall, the distance between eDNA sampling sites throughout the basin ranged from 100–375 m (mean, 221 m).

The sample results showed contractions and patchiness in the distribution of Brook Trout in the system following the 2016 treatment (Figure 6; Table S4). These results were corroborated by electrofishing-based observations of Brook Trout on July 7, 2017, in tributaries 12 and 15 and in the complex habitat of the main stem (near site MS18). Adult Brook Trout were rare, but they were present along with newly emerged fry. Based on the spatial extent of Brook Trout, potential for movement prior to treatment, and minimal additional investment compared with a reduced treatment, a third treatment was conducted on August 15, 2017, which encompassed the same area as the 2016 treatment.
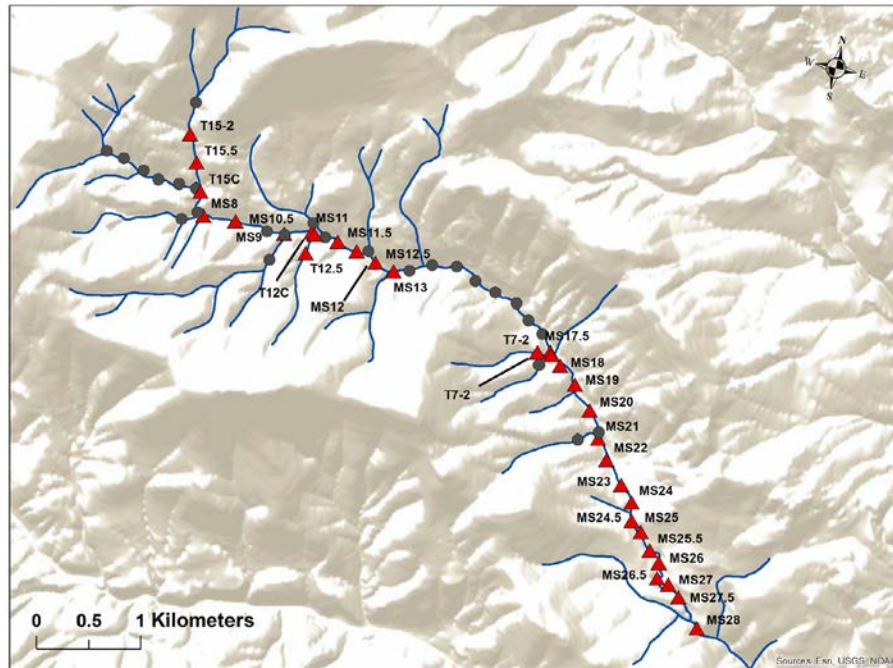
FIGURE 6. Results of eDNA sampling for detection of Brook Trout DNA in Smalle Creek, Washington in June 2017. Red triangles represent sites with positive detections of Brook Trout eDNA; gray circles represents sites where Brook Trout eDNA was not detected. Labels are shown only for sites with positive detections and refer to site ID in Table S4. [Color figure can be viewed at afsjournals.org]

We collected eDNA samples to assess the 2017 treatment between June 4 and 7, 2018, at the same 57 locations that were sampled during the previous year (Figure 7; Table S5). Brook Trout DNA was detected in eight samples–seven in the main stem between sites MS27.5 and MS18 and one in tributary 12 at site T12.5. Between June 25 and 28, 2018, we electrofished the main stem from Smalle Creek Falls upstream to MS17 and the lower 340 m of tributary 12 (the entire treated section of this tributary). No Brook Trout were observed during this survey effort. Additional eDNA samples were collected on June 26, 2018, at MS16, the confluence of tributary 12 (T12-C), T12.5 (200 m upstream of the confluence), and T12-1 (the upper extent of tributary treatment; note that ongoing electrofishing below MS17 occurred downstream of this eDNA sample collection). All four of the samples were negative for Brook Trout DNA (Table S5). Because no live fish and no Brook Trout DNA were detected in or near tributary 12, no further effort was placed on removals in this area of Smalle Creek.

Additional eDNA samples were collected to follow up on positive detections between sites MS27.5 and MS18 in the main stem. On July 17 and 18, 2018, a total of 25 eDNA samples were collected between sites MS28 and MS16 ($n = 23$) and at the mouths of tributaries 4 and 7 (Figure 8; Table S5). To more accurately locate the source of eDNA between MS18 and MS17.5, three

sampling locations were added at 50–70 m intervals between these two locations. Eight of the 25 samples, all located in the main stem from MS25 to MS17.7, were positive for Brook Trout DNA. On July 30, 2018, we intensively electrofished the main stem between MS18 and MS17. Two adult female Brook Trout (both 150 mm total length) were captured in the same large pool 5 m downstream of MS17.6. No fish were observed during a second electrofishing survey of this section on July 31, 2018. On August 1, 2018, we performed an intensive electrofishing survey of the main stem between MS25 and MS22, and no fish were observed during this effort.

On August 7, 2018, a total of four eDNA samples were collected between MS18 and MS17.7 to determine whether DNA persisted throughout this area following the removal of the two live fish. Only the sample that was collected at MS17.7 was positive for Brook Trout (Table S5). Because two fish had recently been removed from this area and because other samples in close proximity were negative, we suspected that the positive detection of Brook Trout DNA at MS17.7 represented DNA that had persisted in the system (e.g., Jerde et al. 2016; Shogren et al. 2017), rather than DNA from a live fish. We decided that an additional treatment was not warranted and waited an additional year to see whether the eDNA would be cleared from the system.
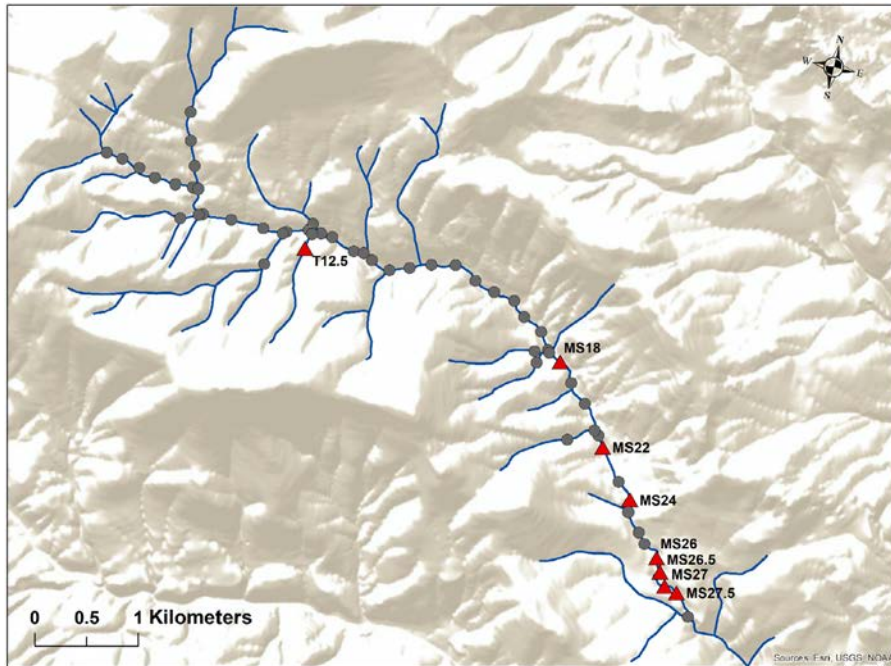
FIGURE 7. Detection of Brook Trout DNA in Smalle Creek, Washington, for eDNA samples collected between 4 and 7 June 2018. Red triangles represent sites with positive detections of Brook Trout eDNA; gray circles represents sites where Brook Trout eDNA was not detected. Labels are shown only for sites with positive detections and refer to site ID in Table S5. [Color figure can be viewed at afsjournals.org]
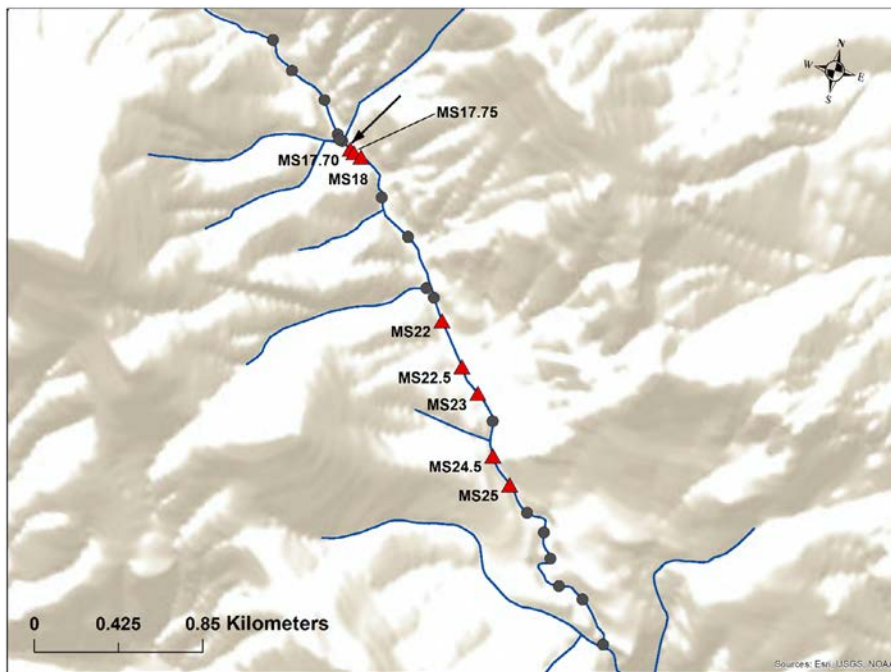

FIGURE 8. Detection of Brook Trout DNA in Smalle Creek, Washington, for eDNA samples collected in July 2018. Red triangles represent sites with positive detections of Brook Trout eDNA; gray circles represents sites where Brook Trout eDNA was not detected. The arrow points to the location between MS17.6 and MS17.5 where two live fish were captured 12 days after eDNA sample collection. Labels are shown only for sites with positive detections and refer to site ID in Table S5. [Color figure can be viewed at afsjournals.org]

On June 5 and 6, 2019, we collected a total of 24 eDNA samples—20 at previously monitored eDNA locations in the main stem between MS28 and MS16.5 and two at new locations between MS25.5 and MS25 where the channel splits temporarily (Figure 9; Table S6). Two samples were collected in tributaries—one each at the confluence of tributaries 4 and 7. Nine samples were positive for Brook Trout DNA including a string of eight detections between MS27 and MS22 and one detection at MS16. On June 28, 2019, we intensively electrofished the main stem between MS27 and MS22 to investigate these positive detections. As with the other previous electrofishing efforts that followed positive eDNA detections of Brook Trout, electrofishing was conducted with the highest settings possible to increase the capture efficiency of live fish and attempt to increase the likelihood of mortality for any fish that were not captured. A single female fish (205 mm total length) was recovered approximately 200 m upstream of MS24.5. No other fish were observed during the electrofishing survey. While no fish were recovered upstream of MS22.5, we noted that the habitat in this section was extremely complex, making it difficult to capture live fish that were persisting in this area.

A final round of eDNA samples was collected on July 8, 2019 at nine locations (Figure 10; Table S5), focusing on the areas upstream of positive detections at MS22.5 and MS16. Four of these samples were collected at a very tight spatial interval in the 130 m upstream of MS22.5 to more precisely determine the location of live fish, if present. All of the samples were negative for Brook Trout DNA. The lack of positive detections in the four samples that were collected upstream of MS22.5 in July 2019 suggested that the source of positive detections at that location in June 2019 may have been located in the 69 m between MS22.5 and MS22.45. Note that an eDNA sample was not collected at MS22.5 in July. Therefore, we cannot be sure whether a fish persisted in this area or the fish had been removed from the area (through volitional movement outside of the sampled area, death and decay, or predation) since the June 2019 detection. Nonetheless, we concluded that any Brook Trout that were persisting in Smalle Creek were present at densities that were too low to repopulate the system. Reintroductions of native Westslope Cutthroat Trout are planned for Smalle Creek in fall 2019 and spring 2020. Environmental DNA sampling may be used to continue monitoring Smalle Creek for the presence of Brook Trout as Westslope Cutthroat Trout recolonize the system.

In the Smalle Creek case study, eDNA sampling was used throughout the entire project to inform the placement and progress of chemical treatments. Fine-scale sample collection towards the end of the project allowed us to target areas for electrofishing to collect and remove surviving fish. Positive eDNA detections in Smalle Creek did not
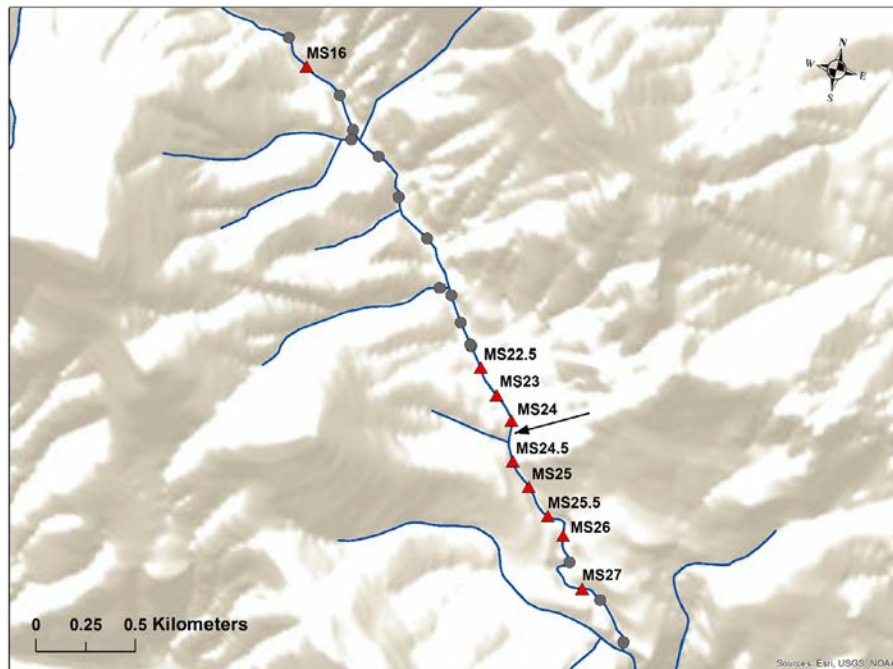

FIGURE 9. Detection of Brook Trout DNA in Smalle Creek, Washington, for eDNA samples collected in June 2019. Red triangles represent sites with positive detections of Brook Trout eDNA; gray circles represents sites where Brook Trout eDNA was not detected. The arrow points to the location between MS24.5 and MS24 where a live fish was captured 3 weeks after eDNA sample collection. Labels are shown only for sites with positive detections and refer to site ID in Table S6. [Color figure can be viewed at afsjournals.org]
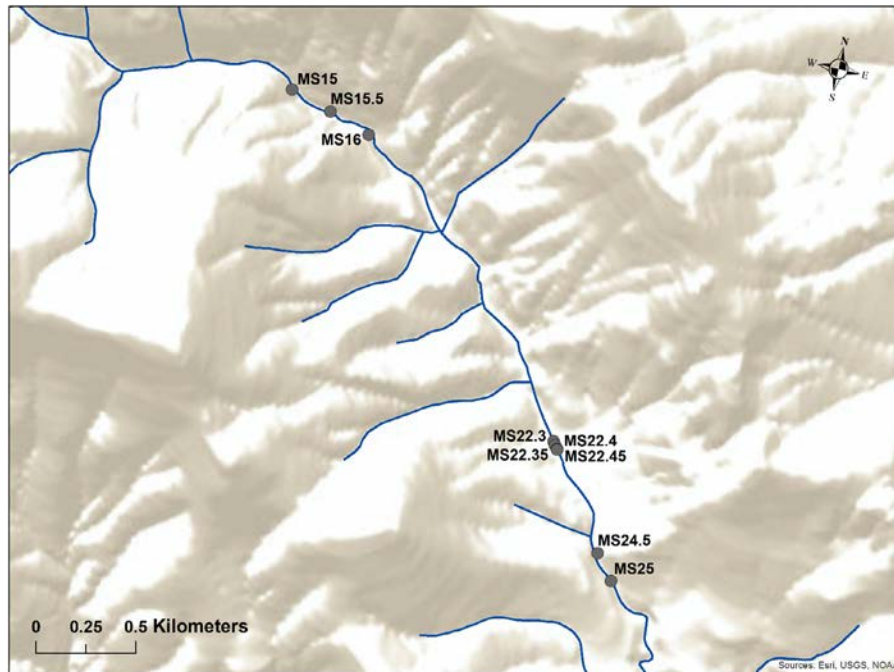
FIGURE 10. Locations of eDNA sampling for detection of Brook Trout DNA in Smalle Creek, Washington in July 2019. Brook Trout eDNA was not detected in any samples. Labels refer to site ID in Table S6. [Color figure can be viewed at afsjournals.org]

always lead to observations of live fish with traditional sampling methods. This is not surprising because eDNA sampling in streams is often more sensitive than are traditional methods for species detection. It is important to note that interpreting these eDNA detections is context specific. Complex habitat in the lower half of Smalle Creek reduced the capture efficiencies of fish and created reaches with higher rates of fine sediment deposition. It is impossible to discern whether uncaptured fish persisted in these areas or increased sediment loads sequestered DNA from previously high densities of Brook Trout. Without the use of eDNA sampling, we would have concluded that the eradication of Brook Trout was complete in 2017 when no live fish were observed with electrofishing. However, persistent positive eDNA detections of Brook Trout prompted further investigation and the recovery of several individuals in following years. Although Brook Trout eDNA was still detected at several locations in the summer of 2019, the quantity of DNA in the positive samples was low and the recovery of live fish was difficult and limited. This led us to conclude that Brook Trout had been suppressed sufficiently to proceed with the next management steps.

DISCUSSION

Any tool that reduces the extent and duration of an eradication project is desirable because it reduces project costs, minimizes mortality of nontarget taxa, enhances public confidence in management actions, and expedites the conservation of native species (Rytwinski et al. 2019). The high probabilities of species detection that were associated with eDNA sampling have made it an obvious and useful addition to many stages of such projects. Environmental DNA sampling, however, is still a relatively new technique (Ficetola et al. 2008). Many questions remain regarding its efficacy under the range of conditions that is encountered by stream biologists. Below, we draw on lessons learned from our case studies and other published studies to provide guidelines for those wishing to use eDNA sampling as part of suppression and eradication projects. This discussion is paired with a decision tree to provide guidance on the use of eDNA sampling in stream-based eradication efforts (Figure 11).

Sampling Objectives and Strategy

The sampling objective, and thus sampling locations, will vary depending on the stage of an eradication project. At the beginning of a project, eDNA sampling can help ensure that the treatment area is comprehensive (i.e., it includes all of the occupied areas, even those with low densities of the target species) but not excessive (i.e., it does not include areas where the target species is absent). Sampling may be directed towards the edge of the target species' distribution while minimizing or even foregoing sampling in areas where the target species' presence is

A Guide for Using eDNA Methods to Assist Invasive Fish Eradications in Streams
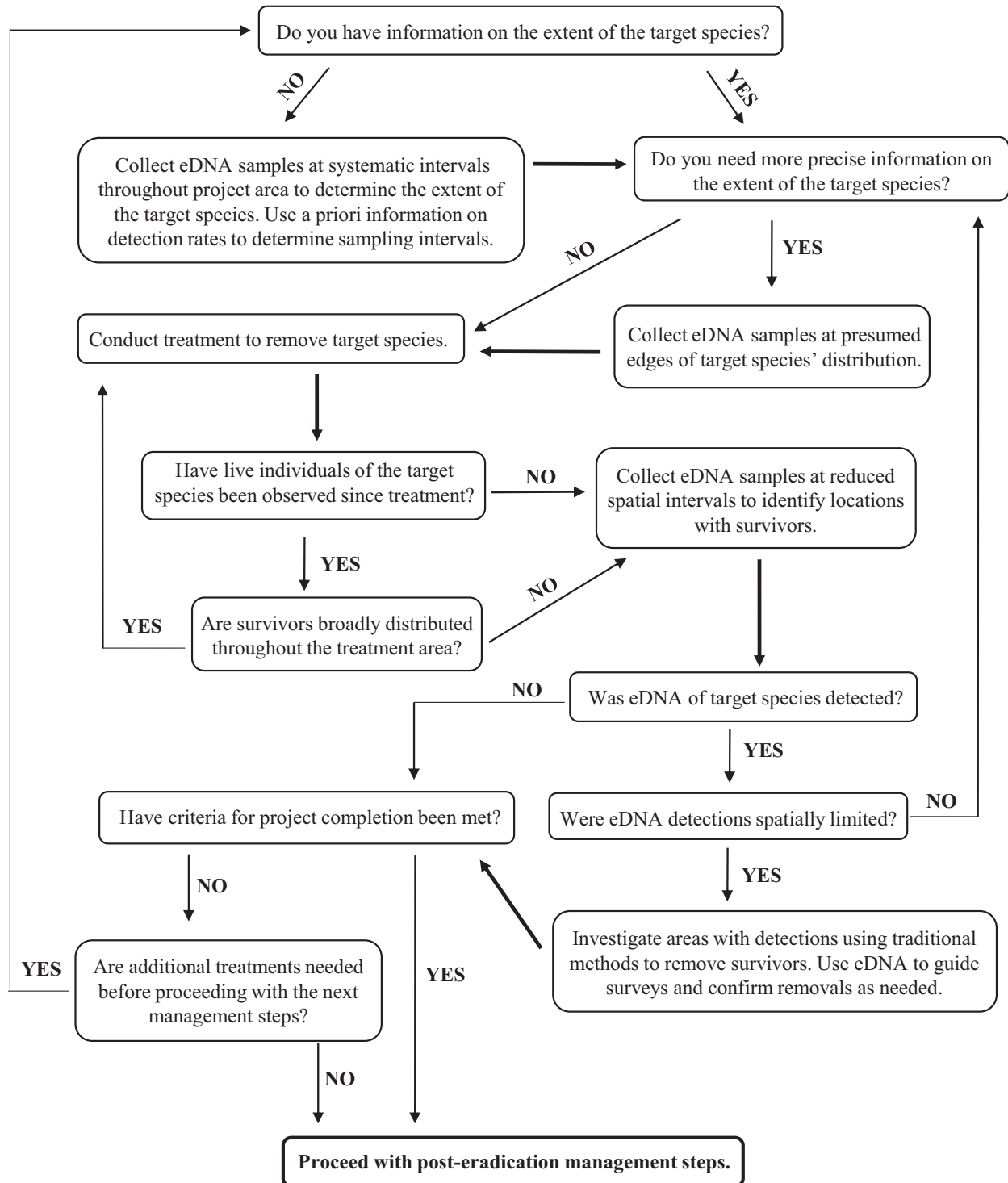

FIGURE 11. A general guide for applying eDNA sampling to inform invasive species eradication efforts in streams. Thicker arrows represent direct transitions to the next step in the guide, while thinner arrows represent one of two transitions based on a "yes" or "no" response. Note that this guide is general and that each project may include unique circumstances that fall outside this decision tree.

known. In a stream setting, eDNA sampling at this stage may target side channels, tributaries, and headwaters. Following one or more treatments, sampling may occur more broadly to identify areas where eradication was successful and direct focus to areas requiring additional treatments. Toward the conclusion of a project, the targeted use of eDNA sampling in tandem with electrofishing can reduce the effort that is necessary to remove any remaining fish while limiting harm to nontarget species as well as verify whether the criteria for successful eradication were achieved.

It is important to note that the optimal sampling intensity depends not only on the stage of an eradication project but also on an array of outside factors including information from other survey methods and abiotic characteristics of the system that is being treated. For example, the densities of stream salmonid populations are typically >10 fish/ 100 m (Young et al. 2005; High et al. 2008; Copeland and Meyer 2011) and the probability of detecting salmonid fish at these densities by using eDNA is high (98.3% at densities ≥2 fish/100 m; Wilcox et al. 2016). Therefore, 1-km sampling intervals can be used to broadly delimit population boundaries (e.g., McKelvey et al. 2016; Dysthe et al. 2018b; Franklin et al. 2019) and define the treatment area during the initial phase of a project. In Smalle Creek, we collected one sample at the highest-elevation drip stations in each tributary and the main stem prior to the first chemical treatment. This information was combined with electrofishing and habitat data to ensure that the treatment area encompassed the distribution of Brook Trout. In systems where less information is available on the distribution of the target species or where a more precise estimate of a species' distribution is needed, it may be necessary to collect multiple DNA samples at tighter spatial intervals. In later project stages (when densities of the target species are lower), sampling intensity should be increased for accurate detection. Following chemical treatments in Greenhorn and Smalle creeks, we collected samples at approximately 250–350 m intervals (following Jane et al. 2015) throughout the treated area to detect any surviving Brook Trout. This sampling interval provided sufficient information to guide next steps. In contrast, flow rate was used to determine spacing between eDNA samples in Sage Creek. This resulted in slightly larger sampling intervals in posttreatment assessments. Several studies have explored the dynamics of eDNA detection and transport as a function of abiotic stream characteristics such as stream flow and sediment sequestration and resuspension (Deiner and Altermatt 2014; Wilcox et al. 2016; Shogren et al. 2017; Pont et al. 2018). However, there is currently no model available for estimating the ideal sampling interval for a particular system, especially when the target species is present in low abundance and habitat is highly heterogeneous. Although DNA may be transported further from its source in large systems (e.g., Deiner and

Altermatt 2014; Pont et al. 2018), most evidence suggests that larger systems will require a higher sampling intensity for the reliable detection of fish (Erickson et al. 2017; Pont et al. 2018; Robinson et al. 2019). This is likely due to the reduced retention and greater dilution of eDNA in larger systems (Jerde et al. 2016; Shogren et al. 2017). A handful of studies have assessed the best eDNA sampling design for eradication projects in lentic systems (Dejean et al. 2011; Dunker et al. 2016; Kamoroff and Goldberg 2018a, 2018b), but many of the factors affecting detection and therefore sampling design are not primary considerations in lotic systems (e.g., unpredictable directional movement of DNA suspended in the water column, retention of DNA sequestered in anoxic sediment, or resuspension of sequestered DNA from turnover).

The optimal sampling strategy will also vary with the life history and ecology of the target species. Focusing sample collection in the preferred habitat of the target species may also increase detection probabilities. For example, one might select different sampling locations for drift-feeding fishes (e.g., salmonids and cyprinids) than for those that live and feed in benthic zones (e.g., larval lamprey and sculpins). Similarly, evidence suggests that the amount of DNA that is produced by individuals may vary with age or life stage (Maruyama et al. 2014) and may be higher during the breeding season (Spear et al. 2015; de Souza et al. 2016; Tillotson et al. 2018). These spatial and temporal differences in a species' distribution can be used to increase detection efficiency by targeting sampling when and where individuals are most likely to be present. To control for habitat heterogeneity throughout a given stream and to maintain objectivity in study design, we recommend sampling at a consistent spatial interval throughout the study area while allowing freedom to add or slightly move sampling locations to further increase the probability of detection based on prior knowledge of the habitat or species distributions in a particular area.

Adjustments may also be made to the materials and protocols that are associated with eDNA sample collection and analysis to improve detection. For example, water volume, filter materials, extraction and analysis methods, and the specificity and sensitivity of the eDNA assays can all influence detection probabilities (Deiner et al. 2015; Dysthe et al. 2018a; Wilcox et al. 2018). While researchers continue to optimize protocols for eDNA sampling across taxa and ecosystems, species detections that use eDNA methods will continue to warrant critical review based on protocols and study design.

Even when the optimal protocols are used, there are some circumstances where traditional sampling methods may perform better than eDNA-based methods to determine a species' presence and distribution. For some species, such as semiaquatic and terrestrial animals that are not constantly in contact with the sampled environment,

traditional survey methods may produce higher detection probabilities than eDNA-based methods (Thomsen et al. 2012; Ushio et al. 2017; Rose et al. 2019; but see Hunter et al. 2015). Furthermore, the advantages of eDNA sampling over traditional methods may vary with the efficiency of traditional methods in a given system. For example, Tréguier et al. (2014) found that eDNA was useful for detecting invasive crayfish in shallow ponds where trapping was difficult but was less effective than funnel trapping in deeper waters. In the case studies presented here, eDNA sampling was effective at detecting live fish in areas with complex habitat that were difficult to sample with traditional methods such as areas with extensive beaver activity, braided channels, and undercut banks in North Fork Sage Creek and lower Smalle Creek. For streams with relatively simple habitat, like Greenhorn Creek, eDNA sampling and electrofishing may have similar detection probabilities. Here, eDNA may be used to focus electrofishing efforts to areas where the target species may persist. Alternatively, managers may simply prefer to proceed with traditional survey methods for a more immediate detection and removal of the target species from the system. Ideally, both traditional and eDNA approaches are used in tandem to take advantage of the strengths of each method.

Identifying and Responding to False Positive Detections

The high sensitivity of qPCR-based analyses to even one copy of DNA (e.g., Mason et al. 2018) adds advantages when looking for low densities of fish in a stream, but it makes eDNA methods vulnerable to providing false positive detections of a target species that arise from non-living or nonlocal sources. We group these sources of false positive detections into four classes: (1) DNA transport by nontarget animals, watercraft, or other vehicles where the animal is not present but its DNA is (Merkes et al. 2014); (2) carcass decomposition (Dunker et al. 2016); (3) release of DNA stored in sediment (Turner et al. 2015; Jerde et al. 2016; Shogren et al. 2017); and (4) contamination during the collection or analysis of samples.

Detections arising from the first of these four sources may occur if a predator transports DNA from a carcass, through feces, or if a boat releases bilge water sourced from a location where the target species was present. These detections should be ephemeral given the rapid decay, transport, and deposition of DNA in most stream systems (Dejean et al. 2011; Pilliod et al. 2014; Dunker et al. 2016), and the rapid clearance of eDNA following the removal of caged animals in several studies (Jane et al. 2015; Robinson et al. 2019). In contrast, carcasses of fish that are killed during treatments may persist for weeks to months (Cederholm and Peterson 1985; Minshall et al. 1991; Chaloner et al. 2002) and can release DNA for at least several weeks (Dunker et al. 2016; Kamoroff and

Goldberg 2018a). Their influence may be reduced by scavengers, macroinvertebrate colonizers, high water temperature and UV light exposure (Merkes et al. 2014; Pilliod et al. 2014), or by intentional removal (as in the Smalle Creek case study). The potential to detect true positives (i.e., eDNA from living individuals) is being explored by sampling for presumably rapidly degrading eRNA (Pochon et al. 2017), but its prospects are uncertain (Cristescu 2019).

The resuspension of DNA from sediment in the water column has been demonstrated experimentally (Jerde et al. 2016; Shogren et al. 2017), but the magnitude of this effect and the duration of in-sediment storage are unknown. In anoxic lake sediments, DNA is abundant (Turner et al. 2015) and can persist for decades or centuries, a property that has been exploited to reconstruct historical patterns of habitat occupancy (Nelson-Chorney et al. 2019). Its long-term persistence in higher energy, well-oxygenated stream environments seems much less likely, given the myriad of factors that contribute to DNA degradation (Barnes et al. 2014). We have chosen to take a conservative position, assuming that DNA may be stored for long periods of time in stream sediments, particularly in stream margins that may be more similar to lentic environments. To minimize false positive detections arising from DNA storage in sediment, we recommend waiting to sample until after a flushing flow or freshet has occurred following the chemical treatment to allow sequestered DNA and/or carcasses to be flushed from the system. Furthermore, sampling during base flow (when levels of suspended sediment are lowest) should increase the probability of detecting a target species, particularly at low densities and on a fine spatial scale. Depending on the timing of treatment and flow regime of a given system, these recommendations may require waiting up to a year before resampling.

Finally, contamination can and will happen from time to time. Negative controls will help identify sources of contamination throughout many aspects of sample collection and analysis, but they cannot always identify contamination arising during the act of sampling itself (e.g., DNA from personal equipment entering the surrounding water and being captured during sample collection). As a result, protocols should be carefully designed and followed to minimize contamination throughout the entire process.

When unexpected results arise (whether positive or negative), the next step should be to consider the sample in the context of the full data set and the local environment. A single positive sample collected in habitat that is ill-suited for the target species or far from other positive detections may more likely represent DNA from a nonliving source. In contrast, a string of positive detections in areas that are known to previously support high densities of the target species may represent surviving individuals or DNA that is sequestered in sediment. In this case, the

quantity of DNA should increase in samples that are collected closer to a live individual. In all cases of unexpected results, we recommend resampling the site and the surrounding area by using eDNA to validate results. Here, detections of live fish should occur consistently and remain relatively high with continued eDNA release (as opposed DNA from nonliving sources, which will degrade over time; e.g., Kamoroff and Goldberg 2018b).

Other Considerations

Although eDNA-based methods are generally more sensitive than traditional survey methods are for detecting fish in streams, they can still fail when animals are at low density or distant from the sampling location. Occupancy models (which require the collection of several independent samples at each site) can be used to estimate the likelihood that an organism is present but is not detected with eDNA-based surveys (e.g., Erickson et al. 2017; Smith and Goldberg 2020). Occupancy models are most useful when there is a significant chance of false negative detection (missing a target individual that is present). Given the high eDNA detection rates of salmonids in small streams, occupancy models are unlikely to further aid management action in stream-based eradication projects. However, for other species, sampling protocols, and waterbodies, occupancy modeling may be useful for integrating eDNA methods into assessments of eradication efforts.

An often overlooked element of eradication projects is defining the criteria for project success (i.e., identifying the point at which removals have been sufficient to extirpate the target population and removal efforts can cease). Obviously, many stream-based eradication projects have been successful without the additional information that is provided by eDNA sampling. It is impossible to know how many of these projects were effective, despite the survival of low numbers of target fish that were unable to reestablish a population. Therefore, the detection of surviving fish with eDNA sampling may not necessarily imply that a removal project has failed. Under such circumstances, continued eDNA sampling can provide evidence that extirpation is eventually achieved. While standards for determining the success of a project may not always change with the increased detection of target species, there are some circumstances where the removal of every individual is necessary. In those cases, eDNA sampling may provide managers with a higher level of assurance when concluding an eradication project.

Summary

Environmental DNA methods can be informative throughout all stages of eradication treatments in stream settings. They can assist with delimiting the population prior to treatment, provide more detailed location data on surviving target individuals, and serve as an efficient and relatively inexpensive monitoring tool to assess long-term treatment efficacy. When used in tandem with traditional survey tools, such as electrofishing, eDNA sampling may help to reduce the size and number of treatments that are necessary to complete an eradication project. This translates directly into increased efficacy of treatments, reduced labor and cost, and reduced adverse effects on the native community. In the later stages of an eradication project, strategic eDNA sampling designs can focus electrofishing efforts (rather than chemical treatments) to remove surviving individuals. This targeted approach further reduces the adverse effects of the eradication project. While a variety of factors may cause false positive and false negative detections, rigorous and carefully followed protocols will reduce their occurrence. Repeated surveys can provide a better understanding of positive detections and provide increased certainty of treatment success. Ongoing research into the retention of eDNA within aquatic ecosystems, variation in detection based on species ecology and abiotic characteristics of the system, and detection rates of various forms of genetic material (e.g., eRNA versus eDNA) will all improve the usefulness of eDNA sampling methods for informing fish eradication efforts.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.