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Using environmental DNA sampling to monitor the invasion of nonnative *Esox lucius* (northern pike) in the Columbia River basin, USA

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Abstract

Aquatic invasive species are recognized as a global threat to conservation of native species and a cost to society. To develop effective suppression and monitoring programs for invasive species, fisheries managers require accurate, affordable, and efficient tools for invasive species detection. In the U.S. Pacific Northwest, the rapid expansion of invasive *Esox lucius* (northern pike) poses threats to native species as well as the viability of tribal, sport, and commercial fisheries. To help monitor changes in the distribution of this species, we developed and rigorously field-tested an environmental DNA (eDNA) assay to detect *E. lucius*. The assay successfully amplified tissue-derived DNA of *E. lucius* from 36 locations east and west of the Continental Divide and did not amplify DNA of over 40 nontarget species. This assay was then used to assist with monitoring the distribution of invasive *E. lucius* in the upper Columbia River basin in Washington and Idaho. Sixty-two eDNA samples were collected at 35 locations of known and unknown *E. lucius* presence. Two samples per site (one on each bank) were collected in larger waterbodies. *E. lucius* eDNA detections were consistent with previous observations of live fish during angler and gill-net surveys, confirming the reliability of the eDNA assay. At two of the 35 sites, only one of the paired samples was positive for *E. lucius* DNA. Varying results between opposite bank samples highlight the need for increased sampling effort when the target species are at low abundance and in large waterbodies. The eDNA assay described in this paper can be used by managers to identify the presence of *E. lucius*, monitor their expansion in western North America, and guide *E. lucius* suppression projects.

KEYWORDS

biological monitoring, Columbia River basin, invasive species, rapid assessment

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1 | INTRODUCTION

Aquatic invasive species are recognized as a global threat to conservation efforts for native species and create an economic cost to society (Gallardo, Clavero, Sánchez, & Vilà, 2016; Lovell, Stone, & Fernandez, 2006). Invasions of nonnative species can have adverse effects on property values, agricultural productivity, public utility operations, native fisheries, commerce, recreation, and ecosystem function (Mefford et al., 2017). In the United States alone, natural resources managers spend $120 billion annually repairing damage from and controlling the spread of invasive species (Pimentel, Zuniga, & Morrison, 2005). In the U.S. Pacific Northwest, the history, culture, recreation, and economy are tightly connected to the production of Pacific salmon and steelhead trout (*Oncorhynchus* spp.). As a result, resource managers and policy makers in the region are concerned about the impacts aquatic invasive species may have on the native fish community.

Esox lucius (northern pike) are native to Holarctic waters in Russia, Europe, Canada, Alaska, and the northeastern United States (Page & Burr, 2011). Their popularity as a sport fish has led to illegal introductions outside their native range, particularly in western North America. *Esox lucius* are considered keystone predators that prefer soft-rayed fish, like salmonids (Craig, 2008). However, they are highly adaptable and can switch their prey when preferred prey densities are low (Eklöv & Hamrin, 1989; Sepulveda, Rutz, Ivey, Dunker, & Gross, 2013). Because of these traits, introductions of *E. lucius* have been linked to declines in native fish populations (He & Kitchell, 1990; Muhlfeld, Bennett, Steinhorst, Marotz, & Boyer, 2008; Ostovar, 2012; Sepulveda, Rutz, Dupuis, Shields, & Dunker, 2015; Sepulveda et al., 2013).

Since the mid-1900s, nonnative *E. lucius* have become established throughout the headwaters of the Columbia River basin, including western Montana and Idaho (McMahon & Bennet, 1996). The first documented sighting of *E. lucius* in Washington was in the Pend Oreille River in 2004 (Harvey, 2011). By 2010, *E. lucius* had spread downstream into the main stem of the Columbia River in Canada and the United States (Lee et al., 2010). In response, the Washington Fish and Wildlife Commission reclassified *E. lucius* as a Prohibited Species in 2011. This classification allowed unlimited harvest of *E. lucius*, required anglers to kill *E. lucius* before leaving the waterbody in which they were caught, and strictly prohibited the transport and release of live *E. lucius* into other waters (Washington Department of Fish and Wildlife, https://wdfw.wa.gov/ais/esox_lucius/). Although suppression efforts have reduced *E. lucius* abundance in the Pend Oreille River (Harvey & Bean, 2017), this species has continued to spread downstream into portions of Lake Roosevelt, a reservoir on the Columbia River in Washington (McLellan, Wolvert, Kittel, Silver, & Lee, in press).

Lake Roosevelt is a National Recreation Area with over 55,000 angler visits per year ([blind coauthor], unpublished data). The fishery is comanaged by three agencies (Confederated Tribes of the Colville Reservation, Spokane Tribe of Indians, and Washington Department of Fish and Wildlife) that have invested $7.4 M annually to protect native fish species and provide harvest opportunities for tribal subsistence and nontribal recreation anglers. The current distribution of *E. lucius* jeopardizes ongoing efforts to protect native salmonids, including Columbia River *O. mykiss gairdnerii* (Columbia River redband trout; Interior Redband Conservation Team, 2016; Jones & McLellan, 2018) and *Salvelinus confluentus* (bull trout), the latter of which is listed as threatened under the U.S. Endangered Species Act (U.S. Fish & Wildlife Service, 1999). If the *E. lucius* invasion expands to the mid- and lower Columbia River, at least 12 other listed stocks *Oncorhynchus* spp. would be at risk (National Marine Fisheries Service, 2016).

Early detection of aquatic invasive species is critical for helping fisheries managers prevent further spread. However, detection with traditional sampling methods (e.g., electrofishing, seining, gillnetting, or angler surveys) over large geographic areas can quickly become cost prohibitive. In addition, sampling with traditional fisheries methods in remote areas can be difficult and time consuming. Under these circumstances, environmental DNA (eDNA) sampling may be a more effective tool for determining the presence and distribution of invasive species like *E. lucius* (e.g., Dejean et al., 2012; Herder et al., 2013; Jerde, Mahon, Chadderton, & Lodge, 2011; Wilcox et al., 2016 [appendix C & D]).

Two eDNA assays are currently published for detection of *E. lucius*. Olsen, Lewis, Massengill, Dunker, and Wenburg (2015), Olsen, Lewis, Massengill, Dunker, and Wenburg (2016) developed an assay in the mitochondrial cytochrome oxidase subunit I (*COI*) gene. This assay was intended for detection of invasive *E. lucius* in Alaska and was not extensively tested for use elsewhere. Spens et al. (2017) designed an assay based on a portion of the mitochondrial cytochrome b (*cytb*) gene. However, this assay was not central to the paper, which was primarily concerned with efficiencies in extraction and storage of DNA. Details concerning its development and testing of the assay are largely absent from the paper. As a result, the reliability of this assay is unknown.

Here, we describe the development of an eDNA assay to detect *E. lucius* throughout their introduced and native range in the United States, including Alaska and locations east of the Continental Divide. Additionally, we evaluated the specificity of previously published assays by Olsen et al. (2015) and Spens et al. (2017) in silico to better understand the appropriate geographic range for each assay and to determine whether they could be paired with our assay for increased accuracy in eDNA results (e.g., Carim, Christianson, et al., 2016c). Finally, we coupled this assay with eDNA sampling across the leading edge of the *E. lucius* invasion in the Columbia River basin. Our goals were to assess the efficacy of eDNA methods for detection of invasive *E. lucius* relative to the traditional methods and to gather additional information on *E. lucius* in areas around the active invasion in the Columbia River basin that are difficult to sample using traditional methods.

2 | METHODS

2.1 | Marker development and validation

We used publicly available data on GenBank to compile DNA sequences of the *cytb* gene of the mitochondrial genome for *E. lucius*

(Table S1) and 15 other species whose ranges commonly overlap with *E. lucius* in western North America (Table S2). We excluded sequences of *E. lucius* from southern Europe in marker development as these fish may represent *E. cisalpinus* (Bianco & Delmastro, 2011; labeled as *E. flaviae* by Lucentini et al., 2011; Table S3) or other members of this genus (Denys, Dettai, Persat, Hautecœur, & Keith, 2014). We used these sequences and the *DECIPHER* package (Wright, 2014) in *R* v. 3.0.1 (R Development Core Team, 2014) to develop a forward and reverse primer set that amplifies 108 bp of the *cytb* gene in the *E. lucius* mitochondrial genome: forward primer 5'-CAGCCACAATCCTCCATTTATTATTC-3'; reverse primer 5'- TGTAGGAGAAGTAGGGATGAAAGGG-3'.

We designed a hydrolysis probe (5'-FAM-CCAGTAGGTAT TAACTCTGATG-MGBNFQ-3') by aligning target and nontarget sequences in MEGA 6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) and identifying a region specific to *E. lucius*. Sequences of *E. masquinongy* (muskellunge; the most closely related species to *E. lucius* in North America) have at least 3 bp mismatches in the probe and both primers (GenBank accessions AY497455AY497456; Table S3). We assessed the melting temperatures of the primers (forward: 59.9°C; reverse: 59.8°C) and probe (69.0°C; including minor groove binder moiety) in Primer Express 3.0.1 (Life Technologies). We screened the primers and probe for secondary structures using IDT OligoAnalyzer (https://www.idtdna.com/calc/analyzer).

The assay was tested using DNA extracted from 51 *E. lucius* tissues collected from 36 different locations across eight U.S. states, as well as 41 nontarget species (Table S4). We selected tissues from nontarget species that were either closely related (e.g., *Esox* spp.), or common in areas where *E. lucius* have been introduced. Tissue-derived DNA was screened in a single 15-µl reaction containing 7.5 µl Environmental Master Mix 2.0 (Life Technologies); 0.75 µl 20X assay mastermix containing forward primer, reverse primer, and probe (with final concentration of 900 nM of each primer and 250 nM of probe per 15 µl reaction); 4 µl DNA template (diluted 1:100 from extracted DNA); and 2.75 µl deionized water. We used cycling conditions of 95°C/10 min [95°C/15 s, 60°C/60 s]×45 cycles on a StepOne Plus Real-Time PCR System (Life Technologies) and viewed PCR results using StepOne Plus software v2.3.

We optimized primer concentrations following methods outlined in Wilcox et al. (2013) for final concentrations of 600 nM per reaction for both the forward and reverse primers (the probe concentration remained at 250 nM). We tested assay sensitivity and identified the limit of quantification by creating a six-level standard curve dilution series (6 250, 1 250, 250, 50, 10, and 2 copies per 4 µl). To create these standards, we cleaned PCR product from the above analysis (*E. lucius* tissue amplified with the assay) using the GeneJET PCR Purification Kit (Thermo Scientific) following the manufacturer's protocol. The standard curve was created by directly quantifying DNA using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and then diluting the DNA in IDTE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) to the desired concentrations. We ran six replicates of each standard dilution using optimized primer concentrations and the cycling conditions above. We used StepOnePlus software v2.3 to estimate the efficiency and R^2 of the standard curve. To determine whether internal positive controls used to assess inhibition affected sensitivity of the assay, we repeated the standard curve analysis but replaced 1.8 µl of water in each reaction with TaqMan™ Exogenous Internal Positive Control (IPC; see below for more details).

To determine the limit of detection and verify accuracy of the standard curve dilutions, we followed methods above to dilute DNA to a concentration of 1 copy per 4 µl and analyzed this standard across 48 replicate reactions. Assuming a Poisson distribution, the probability of obtaining at least one copy of DNA in a reaction at this concentration is 0.63 (see Bustin et al., 2009). As a result, we would expect to detect DNA in about 63% of replicates (approximately 30 of 48).

We screened the assay in vivo using eDNA samples collected from 14 locations across 11 U.S. states where the presence or absence of *E. lucius* was known (Table S5). Samples were collected within 0.5 m of the water's surface by using a peristaltic pump to filter 5 L of water through a 47-mm diameter, 1.5-µm mesh glass microfiber filter (Whatman®) following methods described in Carim, McKelvey, Young, Wilcox, and Schwartz (2016a). Each sample filter was stored individually in silica desiccant at ambient temperature and shipped to National Genomics Center for Wildlife and Fish Conservation (Missoula, MT) for processing. Upon arrival, samples were catalogued and stored at −20°C until extraction occurred.

Environmental DNA was extracted from one half of the sample filter using Qiagen Blood and Tissue DNA Extraction kit with modifications to the manufacturer's protocol and eluted in 100 µl of IDTE (see Carim, Dysthe, Young, McKelvey, & Schwartz, 2016b for more detail). The second filter half was placed in a labeled tube and stored at −20°C for future analysis. Extracted DNA was tested using the PCR conditions with optimized primer concentrations described above, except replacing 1.8 µl of water with TaqMan™ Exogenous Internal Positive Control (1.5 µl 10X Exo IPC master mix and 0.3 µl 50X Exo IPC DNA; ThermoFisher Scientific) to test for PCR inhibition (see below for details on inhibitor detection and removal). All samples were analyzed in triplicate, resulting in a total of 12 µl of eDNA analyzed per sample. A sample was considered positive for the presence of *E. lucius* if DNA was detected in at least one of three PCR replicates.

A sample was considered inhibited if the mean cycle threshold (Ct) for the internal positive control across the triplicate PCRs was delayed ≥ 1 cycle compared to the no-template control. We treated such samples with an inhibitor removal kit (Zymo Research) and reanalyzed each in triplicate. Removal of inhibitors may result in loss of DNA in a sample, but with elution volumes of 100–200 µl, loss of DNA during inhibitor removal is expected to be less than 10% on average (see http://www.zymoresearch.com for more details). To minimize DNA loss during inhibitor removal, we extracted DNA from the second half of the sample filter and combined it with the extract from the first half to obtain ~200 µl of extracted eDNA prior to treatment. The sample was then reanalyzed following the methods above. If the reanalysis showed that a sample was still inhibited after

treatment for inhibitor removal, we analyzed 12 µl of eDNA across four PCR replicates instead of three. Reaction volumes were maintained at 15 µl by increasing the volume of water to compensate for the reduced DNA volume in each of the four replicates. Diluting the amount of DNA in a given PCR by increasing the ratio of water to DNA can also reduce the effects of inhibitors during analysis (McKee, Spear, & Pierson, 2015).

Negative control samples were assessed at multiple stages throughout the protocol to identify any contamination leading to false positive detections. Negative controls were collected in the laboratory and analyzed to test kits containing sampling materials for contamination. One kit per 100 was used to collect a control sample by filtering 0.5 L of DI water as described above. To test for contamination during eDNA extraction, we processed an unused filter from every set of samples extracted (typically one extraction control for every 23 samples). Finally, a negative control was included on each PCR plate to ensure that PCR reagents and laboratory materials were not a source of contamination.

We compared the Spens et al. (2017) *E. lucius* assay to the same 140 *cytb* reference sequences of *Esox* spp. (of which 71 were *E. lucius*) used for in silico development of our assay to identify base-pair mismatches that may affect assay performance (Table S6). Because the Olsen et al. (2015) assay was developed in the *COI* region of the mitochondrial genome, this assay could not be compared to the reference sequences used in development of our assay. Instead, we screened this assay in silico by comparing it to 292 *COI* sequences of *Esox* spp. (of which 154 are *E. lucius*) obtained from GenBank (Table S6).

2.2 | eDNA monitoring of *E. lucius* in the Columbia river basin

We applied our eDNA assay broadly throughout the upper Columbia River basin, from Lake Roosevelt downstream past Chief Joseph Dam, as well as major tributaries including the Kettle River, Colville River, Spokane River, Sanpoil River, and Okanogan River (Figure 1a). This study area spanned the invasion, including areas where *E. lucius* are well established and where they are believed to be absent.

Extensive fisheries research and monitoring surveys have been conducted throughout Lake Roosevelt by its comanagers. This includes year-round angler surveys at 32 access points in Lake Roosevelt resulting in a total of 700 surveys a year (Blake, Kittel, & Nichols, 2017). In addition, the comanagers implement a reservoir-wide annual survey in the first week of November at 150 randomly selected sites using standard gill nets (Blake et al., 2017). Data collected from these surveys have been used to track the expansion of *E. lucius* throughout Lake Roosevelt since they were first detected in 2011. Due to limited funding and difficult access, little fisheries monitoring is conducted in the Kettle or Colville Rivers that drain into the upper reaches of the Lake Roosevelt. To avoid impacts to threatened and endangered fish species, gill-net surveys are rarely used in the main-stem Columbia River downstream of Grand Coulee Dam or in the Okanogan River.

Information from the traditional surveys was used to select 35 eDNA sampling sites. Of these, seven represented sites where *E. lucius* were known to be present and were collected in areas believed to support spawning and rearing. The remaining sites were in areas where *E. lucius* were believed to be absent (n = 20) or were near the invaded area where the presence of *E. lucius* was uncertain (n = 8; Table 1, Figure 1a, Table S7). At each site, we sampled areas with preferred *E. lucius* habitat (slow-moving water and emergent vegetation) and at the mouths of major tributaries to Lake Roosevelt. We treated sites with the same downstream extent but on opposite sides of Lake Roosevelt or tributary rivers as paired sites (n = 27 pairs, median distance between paired sites, 92 m, range 26–2104 m; Table S7). Only one sample per site was collected at locations in Banks Lake and tributary rivers with a wetted width of less than 5 m.

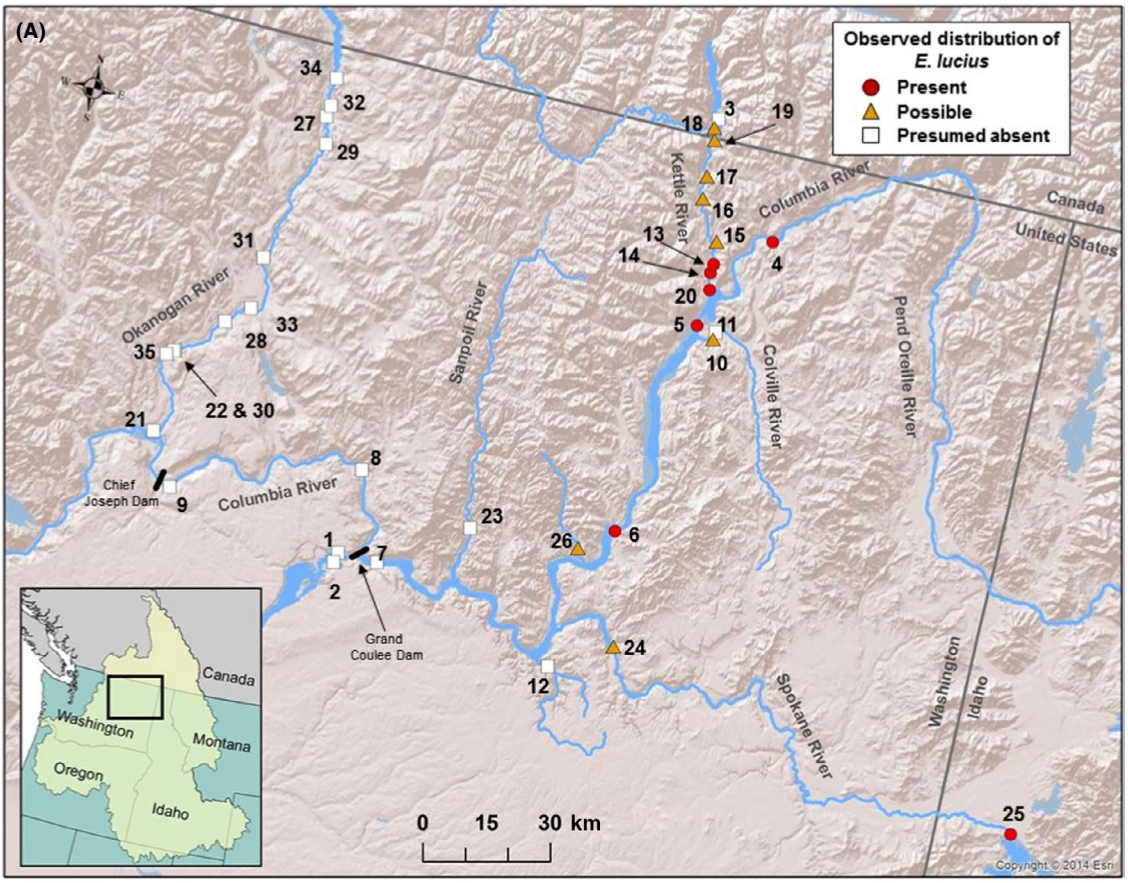
Between 6 September and 6 October 2017, 62 eDNA samples were collected across the 35 sites and analyzed using the methods described above. Samples were collected in fall for two reasons. The hydrograph in the Columbia River basin is driven largely by snowmelt in the spring and early summer. Increased water volume in spring may dilute the DNA of *E. lucius*, decreasing the probability of detection. Additionally, flooding suspends fine sediment in the water column, increasing turbidity. These conditions increase the likelihood of PCR inhibitors in a sample, which may also affect detection rates, particularly when the target species' DNA is present in low quantities. Water samples were collected within the upper 0.5 m of the water column in shallow littoral zones (1–5 m deep) to target the preferred habitat of *E. lucius* (Casselman & Lewis, 1996; Craig, 2008, Dunker et al., 2016; Pierce, 2012).

3 | RESULTS

3.1 | Marker development and validation

Of the 71 *E. lucius cytb* reference sequences obtained from GenBank, eight contained a 1-bp mismatch with the *E. lucius* eDNA assay (Table S1). The location of the mismatch varied, with three sequences

FIGURE 1 Locations of eDNA samples collected in the Columbia River basin for detection and monitoring of invasive *Esox lucius*. Numbers correspond to "Site ID" in Table 1. The Columbia River basin flows westward to the Pacific Ocean and is shown on the inset map in shaded yellow. Lake Roosevelt is a reservoir within the Columbia River, beginning at the border with Canada and extending to Grand Coulee Dam. (a) Observed distribution of *E. lucius*. Sites where live *E. lucius* had been observed prior to sample collection are labeled as "present" (red dots); sites near the invaded area where *E. lucius* presence is possible, but unconfirmed, are labeled as "possible" (yellow triangles); sites where *E. lucius* are believed to be absent are labeled as "not observed" (white squares). (b) eDNA detections of *E. lucius*. Sites with positive detections are labeled as "detected" (red dots); sites where eDNA of *E. lucius* was not detected are labeled as "not detected" (white squares). For sites with paired samples, a location is shown as positive if *E. lucius* DNA was detected in at least one of the two samples


(A)
Observed distribution of
E. lucius
Present
Possible
Presumed absent
Columbia River
Okanogan River
Kettle River
Sanpoil River
Colville River
Pend Oreille River
Spokane River
Chief Joseph Dam
Grand Coulee Dam
Canada
United States
Washington
Idaho
Montana
Oregon
0 15 30 km
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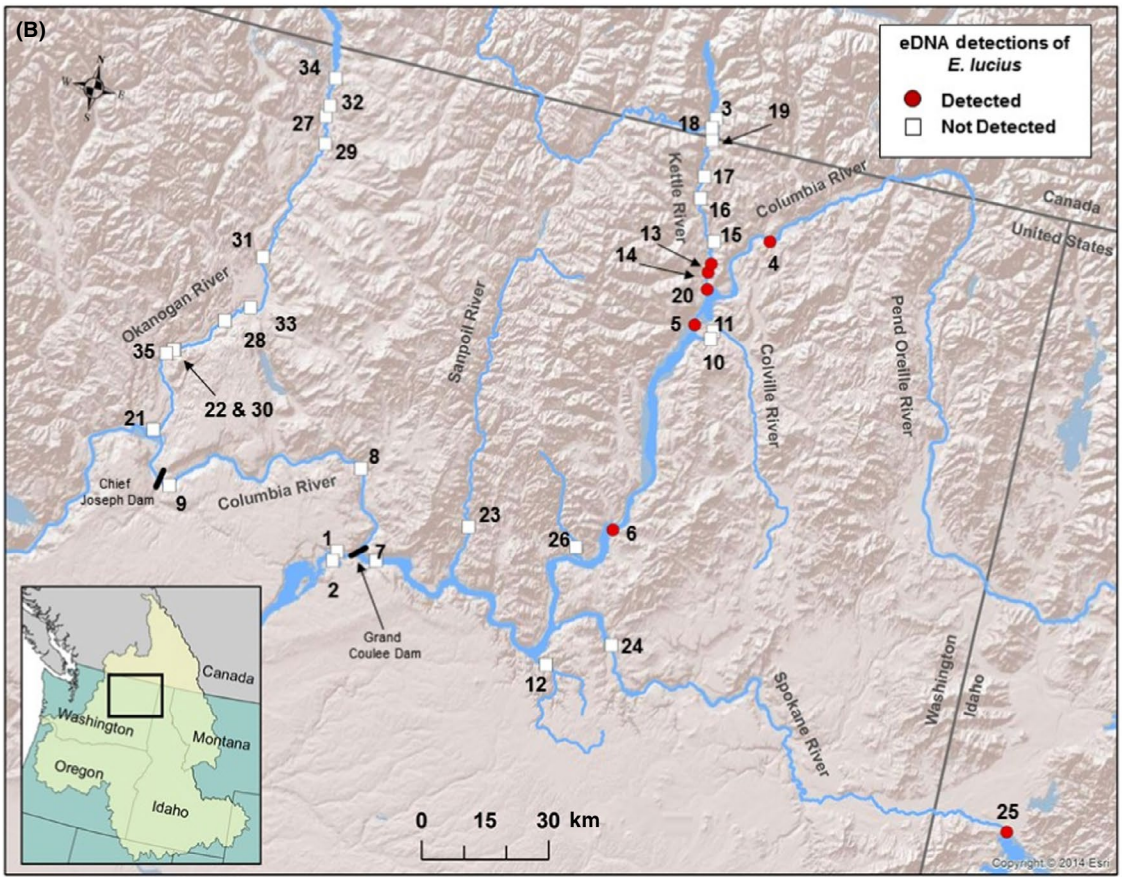
(B)
eDNA detections of
E. lucius
Detected
Not Detected
Columbia River
Okanogan River
Kettle River
Sanpoil River
Colville River
Pend Oreille River
Spokane River
Chief Joseph Dam
Grand Coulee Dam
Canada
United States
Washington
Idaho
Montana
Oregon
0 15 30 km
Copyright © 2014 Esri

containing the mismatch in the forward or reverse primer and five sequences containing the mismatch in the probe. All sequences that were not an exact match to the *E. lucius* assay originated from fish collected in Sweden (n = 1) or Germany (n = 7). Sequences of the *E. cisalpinus* (Lucentini et al., 2011) exhibit up to 4 bp mismatches with the forward primer and up to 2 bp mismatches with the probe (Table S3).

DNA from all *E. lucius* tissue samples successfully amplified, and there was no amplification of nontarget species. Standard curve analysis resulted in an amplification efficiency of 93.9% (r^2 = 0.99, intercept = 38.66, slope = −3.48) and 100% detection across six replicates averaging two copies of DNA per reaction (mean Ct = 38.1). When IPC was included in each replicate of the standard curve, the sensitivity of the assay was unaffected. Here, we observed amplification efficiency of 100.2% (r^2 = 0.99, intercept = 38.79, slope = −3.32) with 100% detection across six replicates with two copies of DNA per reaction (mean Ct = 37.9). Because IPC did not have an effect on the sensitivity of the assay, we did not include it in the PCR test for limit of detection at 1 copy per reaction. We detected *E. lucius* DNA in 33 of 48 PCR replicates with DNA template at 1 copy per reaction (Ct values, 36.6–39.8). These results indicate that our standard curve dilution was accurate and that the limit for detection and quantification of *E. lucius* DNA with our assay is 2 copies per reaction.

In vivo validation of the *E. lucius* eDNA assay was successful. *E. lucius* DNA was detected in all samples collected where this species was known to be present (n = 2); *E. lucius* DNA was not detected in any samples collected in areas where this species was presumed absent (n = 12; Table S5).

In silico comparisons of *E. lucius* assays developed by Spens et al. (2017) and Olsen et al. (2015) indicated that they were potentially less species-specific. The Spens et al. (2017) assay was an exact match to 36 of 40 *cytb* sequences of *E. cisalpinus* in GenBank (Table S6). Similarly, the Olsen et al. (2015) assay was an exact match to 24 of 28 *COI* sequences of *E. cisalpinus* in GenBank (Table S6). Additionally, the Olsen et al. (2015) assay may be less specific because the probe contained no mismatches with 15 sequences of *E. masquinongy* in GenBank. However, this assay may be more sensitive for detection of *E. lucius* DNA, as it was an exact match to all 151 *COI* sequences of *E. lucius* in GenBank. In contrast, both the Spens et al. (2017) assay and the assay developed here contained a 1-bp mismatch with reference sequences of European origin (Tables S1 and S6).

3.2 | eDNA monitoring of *E. lucius* in the Columbia river basin

E. lucius DNA was detected in 9 of 62 eDNA samples collected in the Columbia River basin (Table 1, Figure 1b). The nine positive detections were from the seven locations where live *E. lucius* had been previously observed during angler and gill-net surveys (Blake et al., 2017 and). At two sites, *E. lucius* DNA was detected in only one of the paired bank samples (site 6a, the east bank of Lake Roosevelt near the town of Hunters, WA, and site 25a, the west bank of the Spokane River at the Lake Coeur d'Alene outlet). Additionally, a sample collected on the west bank of Lake Roosevelt near China Bend (site 4b) was positive for *E. lucius* DNA, but results for the sample from the east bank (site 4a) were inconclusive (see below). *E. lucius* eDNA was not detected in samples from 20 locations where this species was presumed absent or 8 locations where presence of this species was possible but unconfirmed.

Two samples showed signs of PCR inhibition: site 4a, at China Bend on the east bank of the Columbia River, and site 20, along the north bank of the Kettle River near Laurier (Table 1, Figure 1b). After treatment to remove inhibitors, *E. lucius* eDNA was detected in the sample from site 20 with no further signs of PCR inhibition. Inhibition was still present in the sample from site 4a after treatment. Subsequent dilution and reanalysis across four PCR replicates did not remove PCR inhibition from this sample, rendering the analysis inconclusive. There was no amplification of *E. lucius* DNA in any equipment or procedural negative controls.

4 | DISCUSSION

4.1 | Assay performance

We have developed and rigorously tested an eDNA assay to assist managers in early detection and monitoring of invasive *E. lucius* in North America. The 71 *cytb E. lucius* sequences used in marker development represented individuals from throughout the Holarctic region, including Canada and Europe. Eight of these sequences from northern Europe were not identical to the eDNA assay. The assay developed by Olsen et al. (2015) may be the best choice for detecting *E. lucius* in northern Europe because its primer and probe are an exact match to all available *E. lucius* sequences from the area. It is, however, prudent to challenge an assay with local samples prior to application in areas where it was not formally tested. The presence of *E. cisalpinus* (Bianco & Delmastro, 2011) and a number of other described and cryptic taxa in the genus *Esox* (Denys et al., 2014; Skog, Vøllestad, Stenseth, Kasumyan, & Jakobsen, 2014) suggests caution in applying any of these assays to waters outside North America.

The sequences of all three assays are identical to all *E. lucius* reference sequences originating from North America, but the Olsen et al. (2015) assay contains no probe mismatches with *E. masquinongy*. Olsen et al. (2015) screened their assay in vitro against one sample of *E. masquinongy* of unknown origin. They did not screen *E. masquinongy* from multiple regions, or test whether higher concentrations of *E. masquinongy* DNA would result in nontarget amplification (see Wilcox et al., 2013). Our assay and that of Spens et al. (2017) have mismatches in both the primers and probes when compared to sequences of *E. masquinongy* DNA. As a result, these assays are a better choice for eDNA applications where *E. lucius* and *E. masquinongy* may co-occur. Of these two, the assay developed here has been tested against all North American *Esox* species, whereas the Spens et al. (2017) assay has not.

TABLE 1 Field data for eDNA samples collected for detection of *Esox lucius* in the Columbia River basin. The "Observed distribution" column indicates the areas of known *E. lucius* presence based on observations of live fish from angler and gill-net surveys as of 2017. Subsequent columns display the results of eDNA sample analysis. There is no Ct value for samples without positive detection of *E. lucius*; larger Ct values generally correspond to lower DNA quantities. Paired samples are labeled with the same map ID and distinguished as either "a" or "b"

Waterbody	Site ID	Site description	Observed distribution	eDNA detected?[a]	No. Positive PCRs[b]	Mean Ct
Banks Lake	1	North bank	Presumed absent	N	0/3	
Banks Lake	2	Near Osbourn Bay, south bank	Presumed absent	N	0/3	
Christina Lake	3a	Lake outlet- east bank	Presumed absent	N	0/3	
	3b	Lake outlet- west bank	Presumed absent	N	0/3	
Columbia River—Lake Roosevelt	4a	China Bend, east bank	Present	ND[c]	ND[c]	
	4b	China Benda, west bank	Present	Y	3/3	40.6789
Columbia River—Lake Roosevelt	5a	Kettle Falls, east bank	Present	Y	3/3	40.3987
	5b	Kettle Falls, west bank	Present	Y	1/3	40.6046
Columbia River—Lake Roosevelt	6a	Near town of Hunters, east bank	Present	Y	2/3	40.8796
	6b	Near town of Hunters, west bank	Present	N	0/3	
Columbia River—Lake Roosevelt	7a	Spring Canyon, west bank	Presumed absent	N	0/3	
	7b	Spring Canyon, east bank	Presumed absent	N	0/3	
Columbia River	8a	Rufus Woods, north bank	Presumed absent	N	0/3	
	8b	Rufus Woods, south bank	Presumed absent	N	0/3	
Columbia River	9a	Chief Joseph Dam boat launch, south bank	Presumed absent	N	0/3	
	9b	Chief Joseph Dam boat launch, north bank	Presumed absent	N	0/3	
Colville River	10	1.6 km upstream of confluence	Possible	N	0/3	
Colville River	11	Below waterfall, 4.2 km upstream of confluence	Presumed absent	N	0/3	
Hawk Creek	12	Approx. 500 m upstream of confluence	Presumed absent	N	0/3	
Kettle River—Kettle River Campground	13	Kettle River Campground, west bank	Present	Y	3/3	35.9210
Kettle River—Napoleon Bridge	14a	Napoleon Bridge, east bank	Present	Y	3/3	35.2480
	14b	Napoleon Bridge, west bank	Present	Y	3/3	37.4584
Kettle River—Barstow Bridge	15a	Barstow Bridge, east bank	Possible	N	0/3	
	15b	Barstow Bridge, west bank	Possible	N	0/3	
Kettle River—Orient Bridge	16a	Orient Bridge, east bank	Possible	N	0/3	
	16b	Orient Bridge, west bank	Possible	N	0/3	
Kettle River	17a	Rock Cut Bridge, east bank	Possible	N	0/3	
	17b	Rock Cut Campground, west bank	Possible	N	0/3	
Kettle River—Laurier	18a	Near town of Laurier, north bank	Possible	N	0/3[c]	
	18b	Near town of Laurier, south bank	Possible	N	0/3	
Kettle River—Cascade Falls	19a	Cascade Falls, north bank	Possible	N	0/3	
	19b	Cascade Falls, south bank	Possible	N	0/3	

(Continues)

TABLE 1 (Continued)

Waterbody	Site ID	Site description	Observed distribution	eDNA detected?[a]	No. Positive PCRs[b]	Mean Ct
Kettle–Columbia Confluence	20	Kamloops Island Campground, east bank	Present	Y	3/3	34.0027
Okanogan River- Mosquito Park, Hwy 97 Bridge	21a	Mosquito Park, Hwy 97 Bridge, east bank	Presumed absent	N	0/3	
	21b	Mosquito Park, Hwy 97 Bridge, west bank	Presumed absent	N	0/3	
Okanogan River—Malott Bridge	22a	Malott Bridge, east bank	Presumed absent	N	0/3	
	22b	Malott Bridge, west bank	Presumed absent	N	0/3	
Sanpoil River	23a	South bank	Presumed absent	N	0/3	
	23b	North bank	Presumed absent	N	0/3	
Spokane River—Porcupine Bay	24a	North bank at Blue Creek confluence	Possible	N	0/3	
	24b	Laughbons Landing, south bank	Possible	N	0/3	
Spokane River—	25a	Lake Coeur d'Alene outlet, west bank	Present	Y	1/3	40.1715
	25b	Lake Coeur d'Alene outlet, east bank	Present	N	0/3	
Wilmont Creek	26	Approx. 3.5 km upstream of confluence	Possible	N	0/3	
Okanogan River	27a	East bank	Presumed absent	N	0/3	
	27b	West bank	Presumed absent	N	0/3	
Okanogan River	28a	East bank	Presumed absent	N	0/3	
	28b	West bank	Presumed absent	N	0/3	
Okanogan River	29a	East bank	Presumed absent	N	0/3	
	29b	West bank	Presumed absent	N	0/3	
Okanogan River	30a	North bank	Presumed absent	N	0/3	
	30b	South bank	Presumed absent	N	0/3	
Okanogan River	31a	West bank	Presumed absent	N	0/3	
	31b	East bank	Presumed absent	N	0/3	
Okanogan River	32a	West bank	Presumed absent	N	0/3	
	32b	East bank	Presumed absent	N	0/3	
Okanogan River	33a	South bank	Presumed absent	N	0/3	
	33b	North bank	Presumed absent	N	0/3	
Okanogan River	34a	West bank	Presumed absent	N	0/3	
	34b	East bank	Presumed absent	N	0/3	
Okanogan River	35a	West bank	Presumed absent	N	0/3	
	35b	East bank	Presumed absent	N	0/3	

[a]Y, detected; N, not detected; ND, inconclusive results.
[b]Number positive PCR replicates/total PCR replicates analyzed; ND, inconclusive results.
[c]Sample treated with Zymo inhibitor removal kit and/or diluted during reanalysis to remove PCR inhibition.

4.2 | eDNA monitoring of *E. lucius* in the Columbia river basin

Environmental DNA monitoring corroborated the known distribution of *E. lucius* in the upper Columbia River basin. We detected *E. lucius* eDNA at all seven locations where *E. lucius* had been previously observed (Figure 1). We did not detect *E. lucius* at any locations where the species was presumed to be absent, indicating that the distribution of *E. lucius* as assessed by eDNA sampling matched that determined by other survey methods. We conclude that the eDNA-based survey described here was as effective at delineating the distribution of invasive *E. lucius* as the combination of a season-long survey of anglers and an intensive gill-net survey. This attests to the accuracy and efficiency of eDNA sampling as a method of invasive species detection at coarse spatial scales.

A limitation of this study is that we did not collect samples in a framework that allowed for robust modeling of detection probabilities at a given site. The probability of detecting a species with eDNA is related to both the abundance and proximity of individuals to the location of sample collection (Pilliod, Goldberg, Arkle, & Waits, 2013; Wilcox et al., 2016). At the two locations where *E. lucius* were detected in only one of two paired bank samples, the species was known to be present at lower densities relative to other locations with consistent detections at both banks (McLellan et al., in press). These results highlight that detection probabilities for a single sample are less than 1.0 when densities are low. This should be considered when attempting to monitor *E. lucius* in areas with even lower densities, such as near the estimated edge of the invasion where the presence of pike is possible but unconfirmed. More intensive spatial sampling in these areas would provide information to estimate detection probabilities of *E. lucius* in a single sample and occupancy at a site scale (Erickson, Merkes, Jackson, Goforth, & Amberg, 2017; Mackenzie et al., 2017).

Our initial work indicates that eDNA sampling is a viable strategy for monitoring the extent of *E. lucius* invasion in reservoirs. We believe that more increased temporal and spatial sampling throughout an area occupied by *E. lucius* could help describe seasonal movements and habitat preferences (e.g., Dunn, Priestley, Herraiz, Arnold, & Savolainen, 2017; Erickson et al., 2016; Erickson et al., 2017; de Souza, Godwin, Renshaw, & Larson, 2016). This information could help maximize suppression efforts by informing where and when they might be most effective. In addition, more extensive sampling outside the known distribution of *E. lucius* would promote a timely response to expansion of *E. lucius* in the Columbia River basin.

4.3 | eDNA strategies for invasive species

Understanding when to use eDNA methods and the best sampling design for detection of invasive species depends on research objectives and data needs. Survey methods that simultaneously detect invasive species and provide an opportunity for suppression (e.g., gillnetting) may be preferred when the leading edge of the invasion can be accurately estimated. This was the case in the upper portion of Lake Roosevelt where ongoing surveys and suppression efforts are underway. In contrast, eDNA methods may be a more efficient tool in areas that are monitored infrequently and where little information is known about the general fish community. In our study, lack of eDNA detections in the Kettle and Colville Rivers informed managers that suppression efforts in these areas would not be the most effective use of limited resources. Intensive spatial sampling may be required when the objective is to more precisely locate the edge of a species' distribution (e.g., McKelvey et al., 2016), or in closed systems where managers wish to determine the occupancy of invasive species following suppression efforts (e.g., Dunker et al., 2016; Davison, Copp, Créach, Vilizzi, & Britton, 2017). Frequent eDNA sampling at sentinel locations may be preferred for early detection of invasive species in areas where species of conservation concern are common. For example, the Okanogan River (which is not yet invaded by *E. lucius*) hosts a number of federally listed fish stocks, including threatened *O. mykiss irideus* (steelhead trout) and endangered *O. tshawytscha* (Chinook salmon; National Marine Fisheries Service, 2017). As a result, the use of gill nets, which lethally sample fish, is prohibited in this area. Nonlethal techniques such as eDNA sampling allow managers to simultaneously monitor invasive and native species while creating a temporal archive of the aquatic community that can be used to inform future management questions.

Some eDNA researchers recommend including a negative field control for each field sample (Goldberg et al., 2016). Protocols for collecting this control sample typically include filtering a small amount of distilled or deionized water in the field. Such field controls are useful when evaluating field protocols and training field crews to collect eDNA samples. Paired negative field controls are imperative in protocols that require field decontamination for pieces of equipment that come into direct contact with every field sample (e.g., a van Dorn sampler). Yet, even these negative field controls will not test for contamination that results from error during field sample collection or from extraneous sources of DNA such as field crews or their equipment (see Merkes, McCalla, Jensen, Gaikowski, & Amberg, 2014; Song, Small, & Casman, 2017). Although we did not include negative field controls (sensu Goldberg et al., 2016), we assessed field contamination by including field samples from areas where the target species is known to be absent. Given the lack of northern pike DNA in these samples, we concluded that field contamination was not a factor influencing the results of this study.

Any field sample with surprising results (whether positive or negative) should be assessed with resampling at the same location (see also Dunker et al., 2016; Wilcox et al., 2016). In our study, suspect samples would be detections in areas distant from known occupied areas or detections in waterbodies representing poor-quality habitat for *E. lucius*. Inconsistent detections over repeated sampling events may represent contamination (false positive detections), degradation, or very low quantities of DNA in the environment (false negative detections; e.g., Erickson et al., 2016), or movement of animals to or from adjacent areas (e.g., McKelvey et al., 2016). Surprising detections should therefore be considered in the context of the larger dataset (e.g., proximity to the nearest

known occupied habitat) as well as movement and habitat preferences associated with the target species' life history. Finally, the most appropriate interpretation of eDNA detections depends on study objectives. When monitoring invasive species, it may be more prudent to interpret positive eDNA detections as the presence of live individuals to ensure a proactive response to introduction and expansion of invasive species (Darling & Mahon, 2011).

5 | CONCLUSIONS

This study has demonstrated the importance of rigorous screening when developing an eDNA assay for broad-scale application. Compared to other published assays, our assay appears to be the most specific and rigorously validated for detection of *E. lucius* in North America. Furthermore, our assay performs as well as traditional survey methods for coarse-scale monitoring of invasive *E. lucius* in the Columbia River basin. Increased spatial and temporal sampling is necessary to inform management on finer spatial scales and to allow for a rapid response to expansion of *E. lucius* in this system. Future monitoring should include sampling at all sites in both the spring and fall to better understand seasonal variation in detection based on life history, seasonal movements, and hydrology.

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AUTHOR CONTRIBUTIONS

J.C.D. and K.J.C. designed the eDNA assay. H.M. and K.J.C. designed the sampling strategy of eDNA sample collection in the Columbia River basin. H.M. oversaw sample collection. K.J.C oversaw laboratory procedures and analyzed results. All authors assisted with data interpretation, writing, and editing the manuscript.

DATA AVAILABILITY

All data presented in this manuscript will be made publically available through the eDNAtlas https://www.fs.fed.us/rm/boise/AWAE/projects/the-aquatic-eDNAtlas-project.html and the National Genomics Center for Wildlife and Fish Conservation.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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