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Detecting spawning of threatened chum salmon *Oncorhynchus keta* over a large spatial extent using eDNA sampling: Opportunities and considerations for monitoring recovery

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Abstract

Over the last century, anthropogenic activities have caused substantial declines in the abundance of chum salmon (*Oncorhynchus keta*) throughout their range in western North America. As a result, chum salmon were listed as threatened under the U.S. Endangered Species Act in 1999. Recovery strategies have been developed, but limited baseline data on distribution impedes implementation of these strategies. Traditional methods for identifying spawning distribution (e.g., spawning ground surveys) may be inadequate for rare species like chum salmon because of a low detection probability. In contrast, environmental DNA (eDNA) sampling is extremely sensitive to species presence and has the potential to supplement or replace existing methods for monitoring listed species. However, legal and administrative issues associated with accurately describing distribution of a listed species put a premium on understanding factors that influence detection of a species by eDNA sampling. In this study, we (a) developed and tested a quantitative PCR-based eDNA assay for chum salmon, (b) collected eDNA samples to describe the spawning distribution of this species in Columbia River tributaries between Bonneville and The Dalles Dams, and (c) tested whether spawn surveyors could inadvertently transport chum salmon DNA between streams on their boots and waders. The newly developed assay was specific and sensitive to chum salmon DNA in both tissue and eDNA samples. Chum salmon DNA was detected in the positive control site and in four streams, which increased known contemporary spawning locations in the study area. In the contamination trial, surveyors successfully introduced chum salmon DNA into the study area but eDNA was only detected intermittently and locally over the next 11 days. The accuracy of eDNA sampling, combined with success in detecting a rare species at the population scale, makes this technique well-suited for monitoring recolonization of chum salmon during the recovery process.

KEYWORDS

assay, distribution, eDNA, *Oncorhynchus keta*, qPCR, recovery, spawning

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1 | INTRODUCTION

Over the last century, Pacific salmon and steelhead have declined throughout the southern portion of their North American range as a result of anthropogenic activities (Nehlsen et al., 1991). Declines have been particularly pronounced in the Columbia River Basin in the northwestern United States, where eight Evolutionarily Significant Units (ESUs) of salmon and five Distinct Population Segments (DPSs) of steelhead are listed as threatened or endangered under the U.S. Endangered Species Act. In response, numerous recovery strategies (e.g., LCFRB, 2010; NOAA, 2013; ODFW, 2010) have been developed to increase the distribution, abundance, productivity, and diversity of these lineages (McElhany et al., 2000). For species present at very low abundances, traditional methods for sampling to evaluate these parameters (e.g., visual spawning surveys to identify distribution) may be inadequate. Consequently, as at-risk taxa become increasingly rare, greater effort may be required to overcome their declining probability of detection while accurately monitoring their status and trends.

In contrast, the extreme sensitivity of environmental DNA (eDNA) sampling, when coupled with quantitative PCR analyses (e.g., Wilcox et al., 2016), suggests that it has the potential to supplement or replace existing methods for some aspects of species monitoring (Franklin et al., 2018; Laramie et al., 2015; Levi et al., 2019; McKelvey et al., 2016; Tillotsen et al., 2018). Studies have shown that eDNA sampling provides accurate, rapid, and spatially extensive data on species presence irrespective of life stage (Dejean et al., 2012) and even at low densities (Wilcox et al., 2016). This sensitivity, however, does come with risks. Although a positive detection of eDNA is interpreted to mean the target species is, or has recently been, present, a false-positive detection might result from transportation of a target species' DNA by a terrestrial predator or via human activities (Merkes et al., 2014; Song et al., 2017), or from cross-reactivity of the assay with a closely related species. The legal and administrative consequences of determining that a listed species is present or absent puts a premium on understanding those factors that influence detection of a species by eDNA sampling.

Chum salmon (*Oncorhynchus keta*) in the Columbia River are an example of a threatened species for which improved monitoring methods are needed. Chum salmon were historically abundant in the lower Columbia River Basin, with estimated annual returns exceeding one million adults (NPPC, 1986). Subsequent declines led to their federal listing (NMFS, 1999) as a single ESU comprising 17 populations (NMFS, 1999). Currently, returns to the Columbia River Basin average 5,000–10,000 adults and are concentrated in two well-sampled populations, Grays River and Lower Gorge (McElhany et al., 2007). For other populations, data on the distribution of spawners are limited. For example, in the Upper Gorge population (bounded by Bonneville Dam and The Dalles Dam on the Columbia River), adult abundance is well characterized because of fish counts at the dams; average counts from 2009 through 2019 were 125 fish/year (range, 21–316; available from Fish Passage Center; https://www.fpc.org/adultsalmon/). Little is known, however, about where these fish spawn because they are rarely observed during spawning surveys.

Chum salmon exhibit an ocean-type life history (Salo, 1991) that makes them particularly well-suited for monitoring using eDNA sampling. In the Columbia River Basin, adult chum salmon primarily return to spawn in the fall (Johnson et al., 1997). Adults spawn in large aggregations in the lowest reaches of streams (Fulton, 1970), suggesting that limited spatial sampling is needed to detect their presence. They may occupy spawning streams for only 1–2 weeks (Lister & Harvey, 1969; Peirce et al., 2011; Rawding & Hillson, 2003) before dying. Thereafter, decaying carcasses may be present in the stream for 2–4 weeks (Rawding & Hillson, 2003) and serve as a substantial source of eDNA (Tillotson et al., 2018). Eggs and alevins remain in the stream gravel until early spring, whence fry emerge and immediately outmigrate (Hale et al., 1985; Salo, 1991). Consequently, the presence of chum salmon DNA at a particular time of year implies presence of a specific life stage.

The goal of our project was to describe the spawning distribution of Upper Gorge chum salmon by using eDNA sampling. To that end, we developed a qPCR assay for chum salmon and collected eDNA samples after peak spawning to compare eDNA-based detections to those from traditional surveys. We also completed a trial study to determine whether fisheries workers contaminated with chum salmon DNA could serve as a source of positive detections. Finally, we conclude by discussing opportunities and considerations for monitoring recovery of chum salmon using eDNA sampling.

2 | METHODS

2.1 | Assay development

We designed the assay in three stages: in silico, in vitro, and in vivo. For the in silico stage, we compiled DNA sequences of the cytochrome oxidase I (COI) mitochondrial gene from the NCBI GenBank database for chum salmon and 14 confamilial nontarget species that are potentially sympatric with chum salmon in western North America (Table S1). This included sequences from chum salmon originating in Alaska (n = 42), Oregon (n = 31), Washington (n = 19), and British Columbia (n = 2), three locations in Russia (n = 30), and in the Sea of Okhotsk (n = 1) in the North Pacific Ocean. There were also three additional chum salmon sequences of unknown provenance in the United States. We aligned these sequences in MEGA6 (Tamura et al., 2013) and screened them for regions that were unique to all chum salmon specimens and distinct from nontarget species. We selected sites that maximized nucleotide mismatches between sequences of chum salmon and the nontarget species to avoid primer competition (Wilcox et al., 2013) and cross-amplification of the probe (Wilcox et al., 2015). We chose primers exhibiting at least six base-pair mismatches between chum salmon and nontarget species (Table S1). The resulting primers amplify a 100-base-pair fragment of the COI gene in chum salmon. Within this amplicon, we designed a FAM-labeled, minor-groove-binding, non-fluorescent quencher (MGB-NFQ) probe (Table 1). We used Primer

TABLE 1 Optimized quantitative PCR assays for detecting chum salmon *Oncorhynchus keta*

Assay component	Sequence (5′–3′)	Tm (°C)	Final concentration (nM)
Forward primer	CCGCTTTTTGTCTGAGCTGTACT	58.8	300
Reverse primer	AATTTCGATCTGTGAGCAACATAGTAA	58.4	900
Probe	6FAM-CTGTACTTCTACTATTATCACTCC-MGBNFQ	69.0	250

Express 3.0.1 (Life Technologies) to evaluate annealing temperatures of the primer and probe and screened them for secondary structures using the IDT OligoAnalyzer web application (https://www.idtdna.com/calc/analyzer). Each oligonucleotide was examined individually using the NCBI nucleotide BLAST tool and NCBI nucleotide collection to identify the potential for detecting nontarget taxa. The primers were also assessed together using Primer-BLAST (Ye et al., 2012) and the full NCBI nucleotide collection.

We then tested the specificity of the assay in vitro by analyzing DNA extracts from 14 chum salmon and 28 nontarget species previously collected by other monitoring projects (Table S2). The DNA used for in vitro screening was obtained from archived samples, or from small fin clips collected from fish that were immediately released at the point of capture. Prior to extraction, we rinsed the tissues with a 10% bleach solution to remove DNA from co-occurring species that may have been on the tissue surface, then thoroughly rinsed each tissue with deionized water. We extracted DNA with the DNeasy Tissue and Blood Kit (Qiagen, Inc.) following the manufacturer's protocol and stored it at −20°C until analysis. The qPCR experiments were performed with a StepOne Plus Real-Time PCR Instrument (Life Technologies) in 15-µl reactions containing 7.5 µl Environmental Master Mix 2.0 (Life Technologies), 900 nM of each primer, 250 nM probe, 4 µl DNA template (~0.1–1.0 ng), and 2.75 µl deionized water. The thermocycler profile contained an initial denaturation stage for 10 min at 95°C followed by 45 cycles of denaturation for 15 s at 95°C and annealing and extension for 1 min at 60°C. All qPCR tests included a no-template control and were prepared inside a hood where plastic consumables were irradiated with UV light for 1 hr prior to set-up. We optimized primer concentrations by varying concentrations of each forward and reverse primer (100, 300, 600, and 900 nM) in triplicate reactions, resulting in 16 unique assay concentrations (cf. Wilcox, Carim, et al., 2015). We selected the assay concentration that displayed a high relative end-point fluorescence and the lowest cycle threshold (Ct) value for use in subsequent analyses (Table 1). Using the optimal primer concentrations, we tested assay sensitivity and efficiency by performing a standard curve analysis created from purified chum salmon qPCR product. This qPCR product was purified using the GeneJET PCR Purification Kit (ThermoFisher Scientific), quantified on a Qubit 2.0 Fluorometer (ThermoFisher Scientific), and then serially diluted to 31 250, 6 250, 1 250, 250, 50, 10, and 2 copies per reaction in sterile TE to create a seven-level standard curve. Each concentration was analyzed across six replicates using the same thermocycling profile described above.

Finally, we validated the assays in vivo by screening six eDNA samples collected from three sampling locations (Table S3). These locations consisted of two sites in which chum salmon were known to be absent and one where they were known to be present based on contemporary survey data. We collected eDNA samples by filtering 5 L of water with a peristaltic pump as described in Carim et al. (2016). We extracted DNA from each filter with the DNeasy Blood and Tissue Kit (Qiagen, Inc.) using a modified protocol (Franklin et al., 2019) which included negative extraction controls. Extracts were stored at −20°C until qPCR analysis. Using the cycling conditions described above, we analyzed eDNA samples in triplicate 15-µl reactions containing 7.5 µl Environmental Master Mix 2.0 (Life Technologies), optimized concentrations of each primer (Table 1), 250 nM probe, 4 µl eDNA extract, a TaqMan Exogenous Internal Positive Control (1.5 µl of 10X IPC assay and 0.3 µl of 50X IPC DNA; Life Technologies), and 0.95 µl of PCR-grade distilled water. All in vivo analyses were run in triplicate and included a no-template control in which distilled water was substituted for DNA. For all qPCR experiments, a reaction was considered positive if it produced a curve with a fluorescence clearly greater than the background signal and thereby crossed the detection threshold during its exponential phase; a sample was considered positive if at least one reaction in the triplicate was positive. A sample was considered inhibited if there was a >1 Ct shift in the IPC relative to the no-template control.

2.2 | Field sampling

We collected eDNA samples from all potential spawning tributaries (active channel width at mouth, 1–150 m, based on satellite imagery; Figure 1; Table 2) entering the Columbia River between Bonneville Dam (at river kilometer [rkm] 235) and The Dalles Dam (rkm 309; Figure 1), for a total of 20 tributaries. Although the presence of chum salmon is unknown in these tributaries, they are inhabited by other salmonid species including coho salmon (*O. kisutch*), Chinook salmon (*O. tshawytscha*), steelhead (*O. mykiss*), and cutthroat trout (*O. clarkii*). To be included in sampling, tributaries had to be accessible to chum salmon (no barriers to migration). Chum salmon typically spawn in the lower-most portions of tributaries (Fulton, 1970), but we included additional upstream samples to verify this distribution pattern. We sampled each tributary longitudinally from its mouth upstream at 1-km intervals until a barrier was encountered (e.g., an impassable culvert or waterfall),

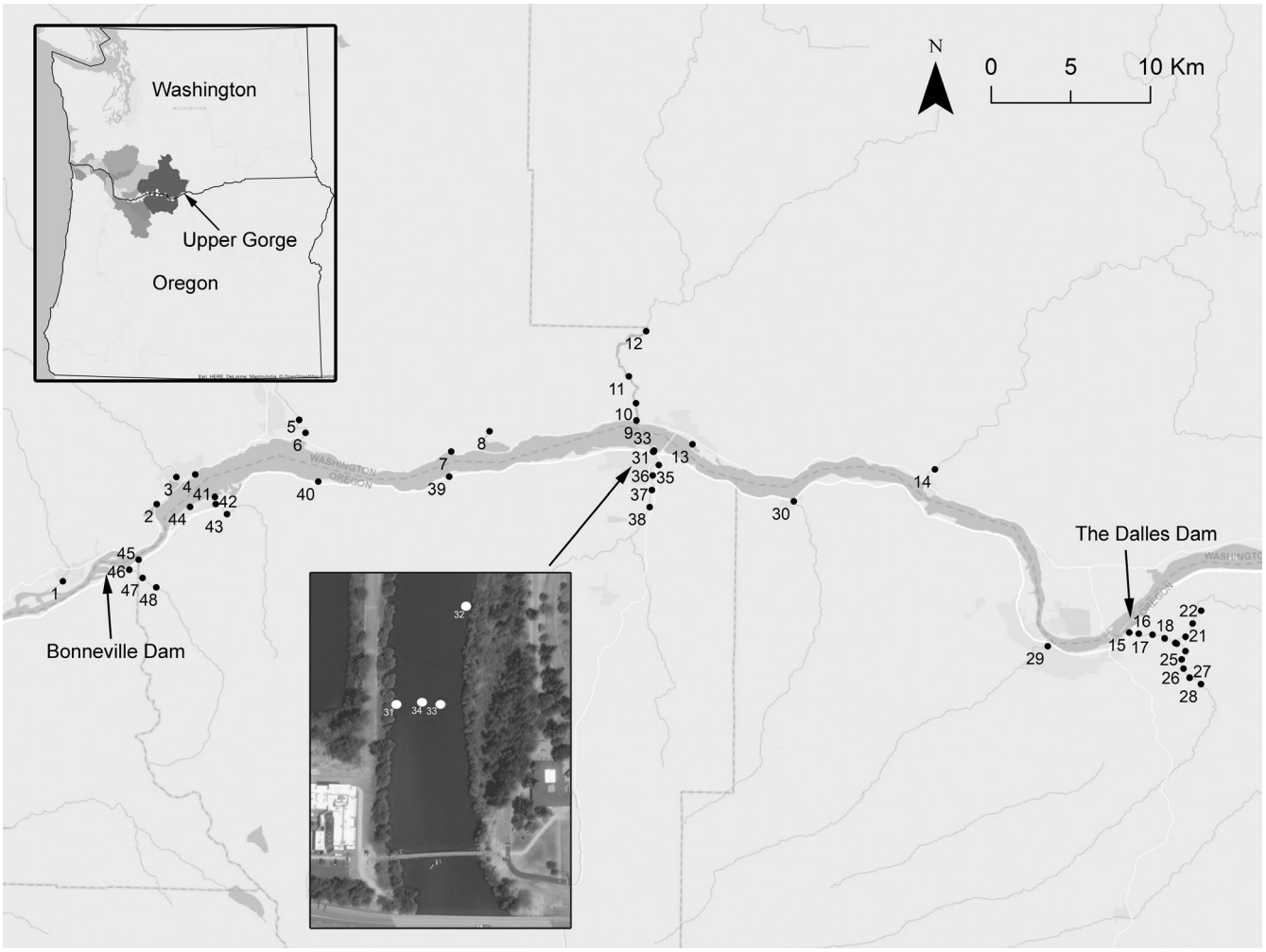

FIGURE 1 Sample sites (with code) within the bounds of the Upper Gorge population in Oregon and Washington where chum salmon*Oncorhynchus keta*environmental DNA samples were collected in 2015. Inset photograph shows Hood River transect sample points 31–34

the stream gradient increased beyond 5% (determined using gradient data from the USDA Coastal Landscape Analysis and Modeling Study in Arc GIS version 10.3.1, ESRI, 2015), or wherever landowners denied access. In 13 of 20 tributaries, only a limited section of the stream was accessible to chum salmon and a single sample was collected near the confluence with the Columbia River. In the Hood River, we collected three additional samples along a cross-channel transect at a point where the active channel width was 75 m. In total, we collected 44 samples from tributaries in the Upper Gorge population. We also collected four samples (also used for in vivo assay tests) from a positive control site in Hamilton Creek on 20 November 2015, a Columbia River tributary below Bonneville Dam that is a known chum salmon spawning area.

We collected the remaining eDNA samples from 21 November to 1 December 2015, and selected sample dates based on the date (11 November 2015) of the peak count of chum salmon at Bonneville Dam. These counts are typically bi-modal, with a few individuals observed in August and September, and the majority seen in late October and November. We began sampling 10 days after peak migration to maximize the potential number of live and dead chum salmon in the study area. All longitudinal sampling was completed in a single day in each stream. Samples were collected in the thalweg in wadable streams, or along a bank in deep rivers. Transect sampling of Hood River was also completed in one day, but 11 days after the longitudinal sampling.

We collected samples in the field according to the protocol developed by Carim et al. (2016). Briefly, a sample was collected by pumping 5 L of stream water through a 1.5-micron, glass microfiber filter using a peristaltic pump. If the stream was turbid and the filter clogged before passing 5 L, additional filters were used until 5 L had been sampled. Filters were preserved in silica desiccant and stored individually in plastic bags. The samples were held at ambient temperature in the field and transferred within 10 hr to a freezer (−18°C). Frozen samples were sent to the National Genomics Center for Wildlife and Fish Conservation (NGC) in Missoula, MT for analysis.

Concurrent with our eDNA sampling, annual surveys to enumerate spawners (hereafter, spawning ground surveys) were conducted by personnel from the Washington and Oregon Departments of Fish

TABLE 2 Sample locations, map code, sample type (longitudinal or transect sample), collection date, presence of chum salmon *Oncorhynchus keta* DNA, active channel width at the sample point, number of qPCR replicates that were positive for detection of chum salmon DNA, average DNA copies per liter, and co-occurrence of spawn surveys for sites where eDNA was collected in the Upper Gorge population of the Columbia River, 2015

Stream	Site code (longitudinal; L, or transect; T)	Collection date	Active channel width (m) at sample point	DNA detected? (# of positive replicates)	Average DNA copies per liter	Spawn surveys conducted?
Ashes Lake N. Trib	2 (L)	11/25/2015	5–10	No		No
Dog Creek	7 (L)	11/29/2015	1–5	No		Yes
Dry Creek	44 (L)	11/22/2015	0–1	No		No
Eagle Creek 1	46 (L)	11/22/2015	25–50	Yes (3/3)	311	Yes
Eagle Creek 2	47 (L)	11/22/2015	10–25	No		Yes
Eagle Creek 3	48 (L)	11/22/2015	10–25	No		Yes
Eightmile Creek 1	23 (L)	11/23/2015	5–10	No		No
Eightmile Creek 2	24(L)	11/24/2015	5–10	No		No
Eightmile Creek 3	25 (L)	11/30/2015	5–10	No		No
Eightmile Creek 4	26 (L)	11/30/2015	1–5	No		No
Eightmile Creek 5	27 (L)	11/30/2015	1–5	No		No
Eightmile Creek 6	28 (L)	11/30/2015	1–5	No		No
Fifteenmile Creek 1	15 (L)	11/23/2015	25–50	No		No
Fifteenmile Creek 2	16 (L)	11/23/2015	5–10	No		No
Fifteenmile Creek 3	17 (L)	11/23/2015	10–25	No		No
Fifteenmile Creek 4	18 (L)	11/23/2015	10–25	No		No
Fifteenmile Creek 5	19 (L)	11/23/2015	5–10	No		No
Fifteenmile Creek 6	20 (L)	11/24/2015	10–25	No		No
Fifteenmile Creek 7	21 (L)	11/24/2015	5–10	No		No

(Continues)

TABLE 2 (Continued)

Stream	Site code (longitudinal; L, or transect; T)	Collection date	Active channel width (m) at sample point	DNA detected? (# of positive replicates)	Average DNA copies per liter	Spawn surveys conducted?
Fifteenmile Creek 8	22 (L)	11/24/2015	5–10	No		No
Gorton Creek	40 (L)	11/22/2015	5–10	No		Yes
Hamilton Creek 1–4	1 (L)	11/20/2015	10–25	Yes (12/12)	45,133	Yes
Herman Creek 1	41 (L)	11/25/2015	10–25	No		Yes
Herman Creek 2	42 (L)	11/25/2015	5–10	No		Yes
Herman Creek 3	43 (L)	11/25/2015	5–10	No		Yes
Hood River 1a	31 (T)	11/21/2015	50–75	No		No
Hood River 1b	32 (T)	12/1/2015	50–75	No		No
Hood River 1c	33 (T)	12/1/2015	50–75	Yes (2/6)	4	No
Hood River 1d	34 (T)	12/1/2015	50–75	Yes (3/3)	224	No
Hood River 2	35 (L)	11/21/2015	50–75	No		No
Hood River 3	36 (L)	11/21/2015	50–75	No		No
Hood River 4	37 (L)	11/21/2015	50–75	No		No
Hood River 5	38 (L)	11/30/2015	50–75	No		No
Jewett Creek	13 (L)	11/29/2015	5–10	No		No
Kanaka Creek	4 (L)	11/25/2015	1–5	No		No
Klickitat River	14 (L)	11/29/2015	25–50	No		No
Little White Salmon River	8 (L)	11/29/2015	10–25	Yes (2/6)	3	Yes
Mill Creek	29 (L)	11/28/2015	5–10	No		No
Mosier Creek	30 (L)	11/28/2015	5–10	No		No
Rock Creek	3 (L)	11/25/2015	25–50	No		No
Ruckle Creek	45 (L)	11/21/2015	1–5	No		Yes
Viento Creek	39 (L)	11/22/2015	1–5	No		Yes
White Salmon River 1	9 (L)	11/30/2015	100–125	No		Yes
White Salmon River 2	10 (L)	11/30/2015	75–100	No		Yes
White Salmon River 3	11 (L)	11/30/2015	25–50	No		Yes

(Continues)

TABLE 2 (Continued)

Stream	Site code (longitudinal; L, or transect; T)	Collection date	Active channel width (m) at sample point	DNA detected? (# of positive replicates)	Average DNA copies per liter	Spawn surveys conducted?
White Salmon River 4	12 (L)	11/30/2015	25–50	No		Yes
Wind River 1	6 (L)	11/25/2015	125–150	Yes (3/3)	171	Yes
Wind River 2	5 (L)	11/29/2015	25–50	No		Yes

Note: Active channel width was estimated from satellite imagery in GIS.

and Wildlife (WDFW and ODFW) in streams throughout the Upper Gorge population. A portion of these spawning ground surveys overlapped with our eDNA sampling and ODFW and WDFW provided survey data for us to compare spawner observations to DNA detections. In Washington, surveys were completed in five streams (n = 27 stream km) every 7–10 days from 5 August to 4 December 2015 (Brad Garner, WDFW unpublished data). These surveys targeted Chinook salmon, but observations of other species, including chum salmon, were also recorded. In Oregon, surveys were completed in eight streams (n = 11.97 stream km) every 7–10 days from 15 October to 31 December 2015 (Ryan Jacobsen, ODFW unpublished data). These surveys targeted coho salmon, but chum salmon observations were recorded. All survey reaches in Washington and most of those (10.93 km) in Oregon were in streams that were part of the eDNA survey (Figure 1; Table 2).

2.3 | Laboratory methods

Upon receipt of samples at the NGC, we catalogued the sample data and stored the samples at −20°C until analyzed. The samples were extracted and analyzed following the optimized methods, experimental preparation, and thermocycling conditions noted earlier with two exceptions. First, negative controls were accidently excluded during eDNA extraction of 2015 samples. However, results suggest that contamination introduced during eDNA extraction is not a likely source of DNA in these samples (see results below). Second, during qPCR analysis, we included positive controls containing approximately 0.4 ng DNA extracted from chum salmon, and negative controls containing molecular-grade distilled water in the place of DNA template in each analysis. Consistent with methods for field samples, these controls were analyzed in triplicate. Whether a detection was positive, or was inhibited, was interpreted as noted above. If a sample was deemed to be inhibited, DNA was extracted from the second half of the filter and DNA from both filter halves was combined and cleaned by running through an inhibitor removal column (Zymo Research; www.zymoresearch.com) and then re-analyzed.

2.4 | Contamination trial

To explore whether individuals conducting spawning ground surveys could inadvertently contaminate a stream and lead to false-positive detections of chum salmon, we conducted an intentional contamination experiment by introducing chum salmon DNA to a small stream. The contamination trial was conducted in Mill Creek, a 2nd-order tributary to Big Creek in the lower Columbia River basin (average width 1.5 m, average depth 0.25 m). Mill Creek is a water source for Big Creek Hatchery and there is a barrier 5 m upstream of its confluence with Big Creek that prevents fish from entering the creek. The substrate is comprised of large gravel and small cobble (4–90 mm). The hydrograph is rain-dominated, with rain-driven floods typically

in fall and winter. Water height data (recorded as water pressure; kilopascals, kPa using a model U20 Hobo water level logger by Onset) were collected in the creek at the downstream sample site to describe the timing of flow events.

On 14 November 2016, eDNA samples were collected to confirm that no chum salmon DNA was present in the system. On 17 November, a carcass from an adult chum salmon was placed in a rubber tote filled with approximately 30 L of stream water and placed on the stream bank, 0.5 m from the active channel. A "surveyor" wearing canvas waders and felt-soled boots stepped into the tote and swished the water around for 1 min, then stepped directly from the tote into Mill Creek, taking care to not step on the bank. Once in the creek, the surveyor walked upstream 10 m and then stopped. At this point, a second individual collected an eDNA sample at a location 1 m downstream from where the surveyor had entered the water (hereafter, the "contamination" site). Once that sample was collected, the surveyor walked back to the entrance point in the stream, stepped into the tote, and then stepped from the tote up onto the bank above the bankfull elevation. This was done to ensure that DNA would not be deposited outside of the creek in an area that could flood during the study and introduce additional DNA to the creek. A second eDNA sample was collected at the contamination site 10 min after the first sample. Finally, an eDNA sample was collected 75 m downstream 30 min after contamination. The tote containing the carcass and contaminated water were carried to Big Creek Hatchery and emptied. Every day for the next 11 days, a sample was collected at the contamination and downstream sites, beginning at the downstream site first. On day 15 (1 December 2016), one last sample was collected at each site.

3 | RESULTS

3.1 | Assay development

The sequences of the forward primer, reverse primer, and probe were identical in all chum salmon sequences available in reference databases (Table S1). Further, the assay amplified DNA from all chum salmon tissue samples and did not amplify DNA from nontarget species or any negative controls. The standard curve demonstrated a reaction efficiency of 107.10% (r^2 = .995, *y*-intercept = 36.375) and a limit of detection (defined here as the lowest concentration with >95% amplification success; Bustin et al., 2009) of 10 copies per reaction; DNA was detected in all six replicates at this concentration and in two of six replicates averaging 2 copies per reaction. During in vivo validation, chum salmon DNA was not detected in either environmental sample taken where the species was expected to be absent and was detected at the site where the species was known to occur (Table S3).

3.2 | Spawning distribution

In 2015, 176 chum salmon were detected passing over Bonneville Dam between 10 September and 24 November (Figure 2). Most (n = 168) passed within a month of sampling (peak count, 11 November) and had the potential to be present in spawning streams (either alive or as carcasses) prior to sampling. Four chum salmon passed over Bonneville Dam before 21 October and four passed over during the eDNA sampling period and may not have available for detection by eDNA sampling.

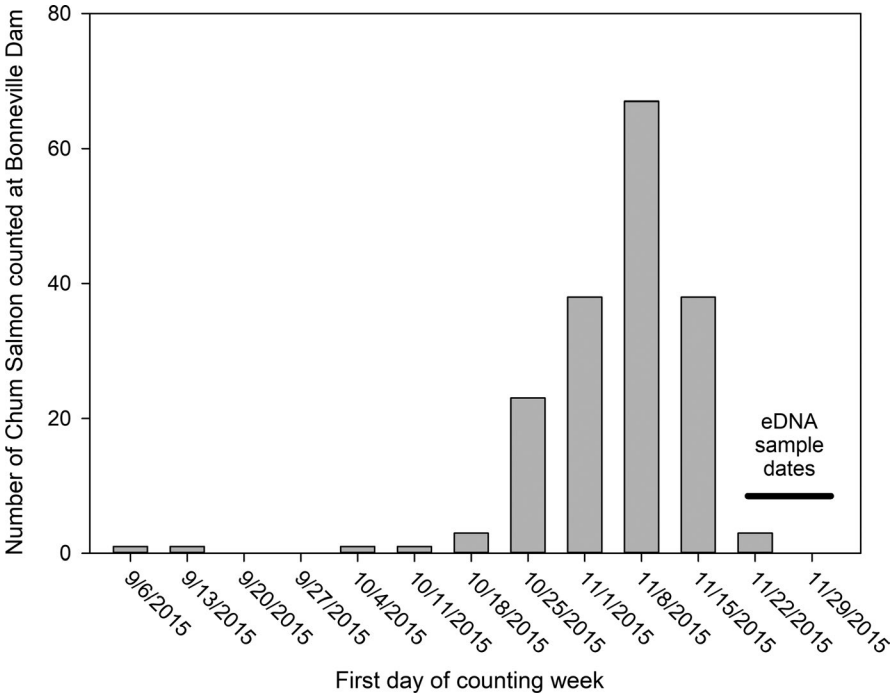

FIGURE 2 Weekly totals of chum salmon*Oncorhynchus keta*passing over Bonneville Dam on the Columbia River in 2015. The eDNA sampling dates (21 November–1 December) are labeled with a black line

We detected chum salmon DNA in the four positive control samples collected from Hamilton Creek, and in four of the other 20 study streams: Eagle Creek, Hood River, Little White Salmon River, and Wind River (Table 2). These positive detections occurred in streams with active channel widths ranging from 10 to 150 m. In the Hood River, chum salmon DNA was only detected on the later sample date and at two of the three transect points (Figure 1; Table 2). In the other three streams, chum salmon were only detected at the downstream-most sample locations. None of the equipment and laboratory control samples were positive for the presence of chum salmon eDNA and inhibition was not detected in these samples. While extraction controls were not included during analysis of these samples, lack of chum salmon DNA in a majority of these samples indicates that contamination during extraction was not a likely source of DNA in this dataset.

Chum salmon (n = 3) were observed during spawning ground surveys only in the White Salmon River, Washington, well before eDNA sampling began. The first two observations (one live fish and one carcass) were on 28 September 2015 and may have represented the only two chum salmon that had passed Bonneville Dam (on 6 and 13 September) by the time of their observation. The third live fish was seen on 26 October, at which point nine chum salmon had been detected at Bonneville Dam. The remaining 173 chum salmon potentially present in the Upper Gorge population were not observed on spawning ground surveys.

3.3 | Contamination trial

The eDNA results demonstrate chum salmon DNA was not present in the creek prior to the experiment nor in any of the negative controls. After intentional contamination, chum salmon DNA was detected in the samples collected at the contamination site immediately after contamination and 10 min later, and at the downstream site 30 min later (Figure 3; Table S4). A limited quantity of chum salmon DNA was also detected on days 4 and 11, but only at the contamination site (Figure 3; Table S4). On days 3 and 10, storm events caused flows to increase, and the second event caused an increase in turbidity that temporarily precluded sampling because the excessive sediment rapidly clogged the filters. Neither rain event caused the creek to inundate the bank near the contamination site. Both subsequent detections of chum salmon DNA occurred as the

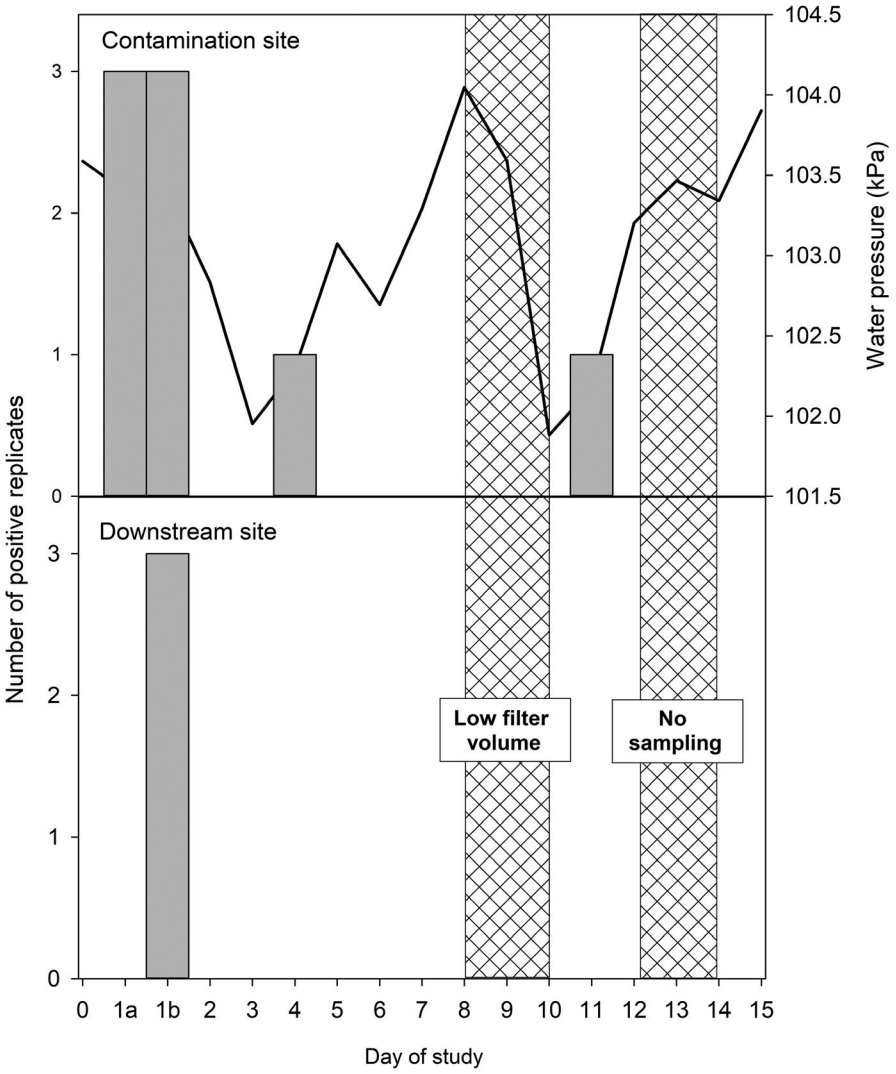

FIGURE 3 Number of replicates where chum salmon*Oncorhynchus keta*DNA was detected from each water sample and corresponding water height (recorded as pressure; kPa) during sample events. Three samples were collected on the contamination day (day 1): during (1a) and 10 min after contamination (1b) at the contamination site, and 30 min after contamination at the downstream site (1b)

hydrograph began to rise but did not continue throughout the period of elevated flows (Figure 3).

4 | DISCUSSION

In this study, we successfully developed and validated a new eDNA assay for chum salmon. The qPCR assay reliably and sensitively detected chum salmon DNA at concentrations below 10 copies per reaction. Although the assay was tested in vivo only in the Columbia River basin, we expect this assay to be sensitive to the presence of chum salmon anywhere in western North America because of its generality among all chum salmon sequences in public databases. It is also specific to chum salmon DNA because it did not amplify DNA from any other member of *Oncorhynchus* in this region or of other common sympatric taxa. As such, eDNA sampling for chum salmon should be highly sensitive when paired with proper study design and robust protocols (Wilcox et al., 2018).

Using the new assay, we conducted eDNA sampling for chum salmon within potential spawning areas in the Upper Gorge population and detected DNA in four of the 20 sampled streams. Contemporaneous spawning ground surveys failed to detect the presence of chum salmon in any of these streams. Because chum salmon are rarely observed during spawning surveys in the Upper Gorge, these detections using eDNA sampling represent a new baseline distribution against which future recovery actions can be compared. Results from longitudinal sampling suggest that only the lowermost portions of any of the study streams may have been occupied at the time of sampling. This is consistent with the understanding that chum salmon spawn in the most downstream reaches where low gradient habitat occurs (Fulton, 1970).

In rapidly evaluating spawning distribution on only 1–2 days in each stream, it is possible that some spawning fish were not detected. This could occur if chum salmon appeared in a stream well before or after we sampled. Likewise, if fish were rare and locally distributed, sampling on a single bank could be insufficient to detect a localized plume of DNA, particularly where the stream is not well mixed (Laporte et al., 2020; Wilcox et al., 2015; Wood et al., 2020). However, repeated (Erickson et al., 2019; Goldberg et al., 2016) or more spatially comprehensive sampling (Goldberg et al., 2015) should increase the probability of detection in such scenarios.

Alternatively, it is possible that eDNA detections represented false positives if anglers or surveyors inadvertently transported chum salmon DNA among streams on their waders and boots. Although we cannot reject this possibility using data from a single sample event, we believe it is unlikely for three reasons. First, angling for chum salmon is prohibited in the Columbia River and because of their low abundance, they are rarely observed in streams where anglers target other species. The low-level detections in our contamination trial were produced from a heavily contaminated surveyor in direct contact with a carcass, not consistent with how an angler might land and release a fish. Second, it is extremely unlikely that Oregon spawn surveyors could have serve as a source of chum salmon DNA because they did not directly contact or even observe any during their surveys. Washington spawn surveyors encountered chum salmon in September and October on the Little White Salmon River, but not on any other repeat surveys or in any other streams during the rest of the run. If the October contact with chum salmon introduced DNA into the Wind River at that time, results from our contamination trial suggest that the DNA would no longer be detectable by the time we collected our water samples a month later. Finally, field crews conducting eDNA sampling took precautions to avoid contaminating sites or samples, including wearing new waders and boots. For these reasons, it appears that eDNA detections in the Upper Gorge were more likely produced by fish than by contamination. However, if eDNA surveys were focused on a more abundant or harvestable species, the potential for contamination would have to be explicitly incorporated into the sample design.

False-positive detections of species have been considered a drawback of eDNA sampling (Evans et al., 2017; Rees et al., 2015), yet the contamination trial reaffirmed that detections of indirectly introduced DNA were generally limited in both extent and duration. This is unsurprising, in that a number of caged animal studies have repeatedly demonstrated clearing of DNA from sample streams in less than 24 hr (Jane et al. 2015; Pilliod et al., 2014; Robinson et al. 2019). The detections following storm events, however, indicate longer-term storage and resuspension of eDNA in the water column is possible (Stoeckle et al., 2017), albeit localized and of limited quantity (i.e., inconsistent amplification across qPCR replicates). These results imply consideration of sample size, site selection, and repeat sampling, particularly in relation to rain events, may be sufficient to overcome potential effects of surveyor contamination. Further testing in a range of environments is required to determine the prevalence of surveyor contamination and to identify decontamination protocols for boots and waders that would be feasible in a field setting.

In conclusion, eDNA sampling provided valuable baseline data on the contemporary spawning distribution of chum salmon from the Upper Gorge population, identifying chum salmon presence in streams where traditional survey methods had failed to detect them. We suspect that eDNA sampling might also provide information about other portions of the life cycle (cf. Laramie et al., 2015). In this study, we inferred that positive detections of DNA were produced by live and dead adults associated with spawning activities. Extending this sampling to periods of egg incubation, juvenile emergence, and outmigration could reveal critical aspects of the ecology of this species. Elsewhere, eDNA sampling has been used to detect the presence of juveniles following their outmigration to estuaries (Minegishi et al., 2019). In the face of ongoing declines and attempts at reintroductions (NOAA, 2013; ODFW, 2010), such information is of critical importance to stakeholders involved in recovery actions.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

KMH, KJC, TWF, and MKY conceived of and designed the study. KMH collected field samples and provided tissue samples. JCD processed genetic samples. KSM, JCD, and TWF analyzed genetic results. KMH and TWF drafted the manuscript. MKY improved the manuscript. All authors edited the manuscript and approved this final version.

DATA AVAILABILITY STATEMENT

Raw data from this study are archived at the Oregon Department of Fish and Wildlife, West Region office, by the Chum Reintroduction Coordinator.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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