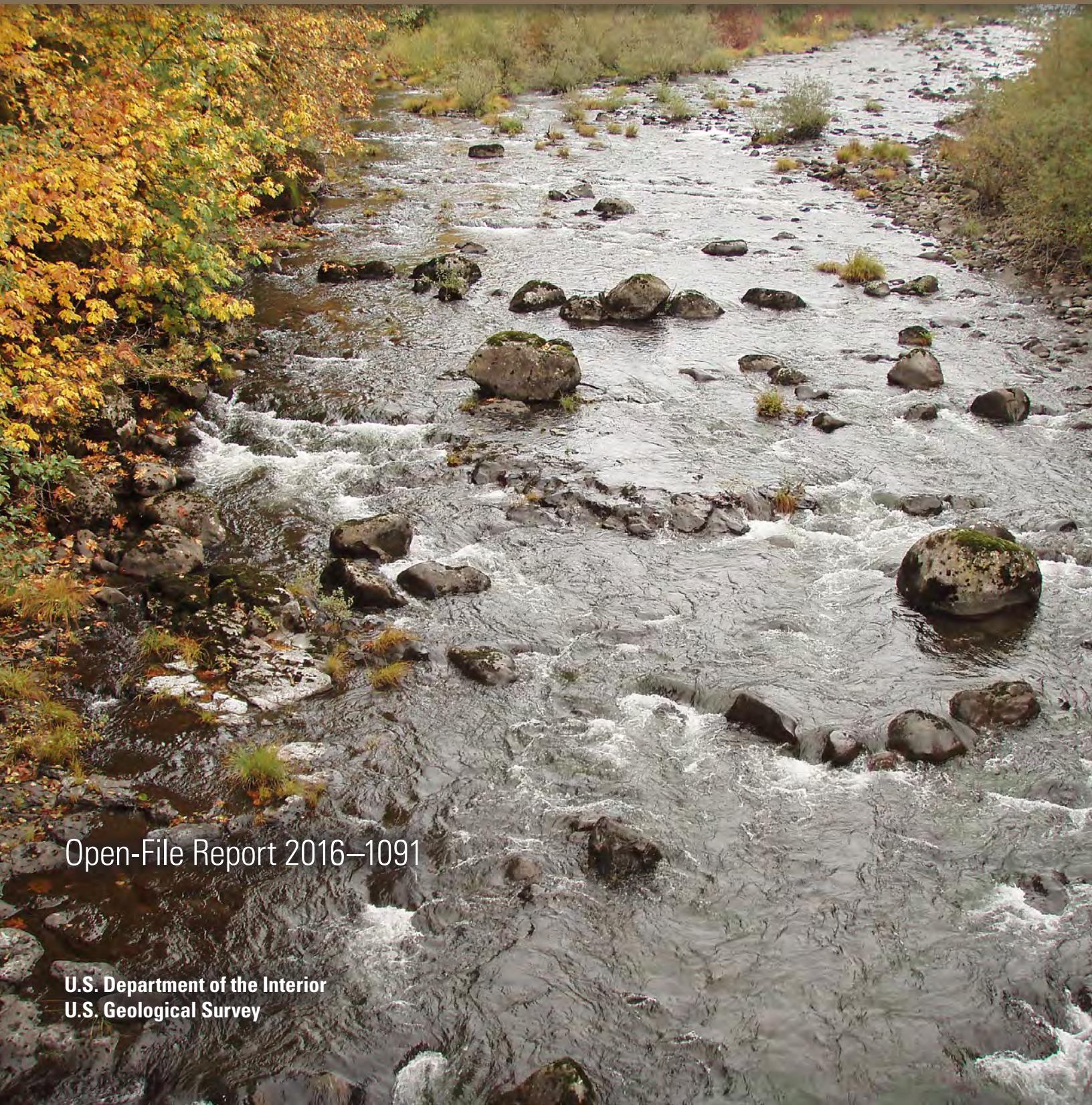


Prepared in cooperation with Portland Water Bureau

# Salmon Redd Identification Using Environmental DNA (eDNA)



Open-File Report 2016–1091

**Cover:** Photograph of salmon spawning area in the Bull Run River in the Sandy River Basin, Oregon.  
Photograph by Burke Strobel, Portland Water Bureau, October 19, 2009.

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By David S. Pilliod and Matthew B. Laramie

Prepared in cooperation with Portland Water Bureau

Open-File Report 2016-1091

**U.S. Department of the Interior  
U.S. Geological Survey**

**U.S. Department of the Interior**  
SALLY JEWELL, Secretary

**U.S. Geological Survey**  
Suzette M. Kimball, Director

U.S. Geological Survey, Reston, Virginia: 2016

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## Conversion Factors

### Inch/Pound to International System of Units

Multiply	By	To obtain
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)

### International System of Units to Inch/Pound

Multiply	By	To obtain
kilometer (km)	0.6214	mile (mi)
milliliter (mL)	0.033814	fluid ounce (fl oz)

Temperature in degrees Celsius ( $^{\circ}\text{C}$ ) may be converted to degrees Fahrenheit ( $^{\circ}\text{F}$ ) as  $^{\circ}\text{F} = (1.8 \times ^{\circ}\text{C}) + 32$ .

## Datum

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

# Salmon Redd Identification Using Environmental DNA (eDNA)

By David S. Pilliod and Matthew B. Laramie

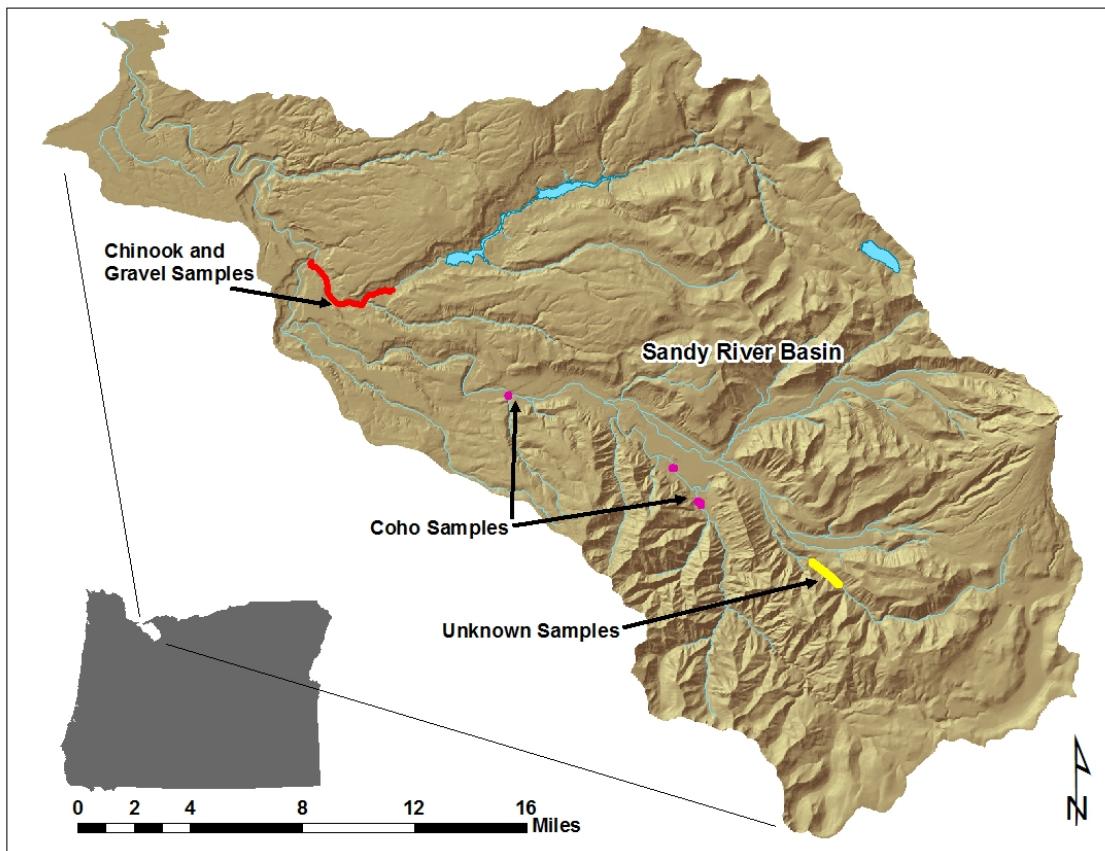
## Introduction

The purpose of this project was to develop a technique to use environmental DNA (eDNA) to distinguish between redds made by Chinook salmon (*Oncorhynchus tshawytscha*) and redds made by Coho salmon (*O. kisutch*) and to distinguish utilized redds from test/abandoned redds or scours that have the appearance of redds. The project had two phases:

- Phase 1. Develop, test, and optimize a molecular assay for detecting and identifying Coho salmon DNA and differentiating it from Chinook salmon DNA.
- Phase 2. Demonstrate the efficacy of the technique.
  - a. Collect and preserve water samples from the interstitial spaces of 10 known redds (as identified by expert observers) of each species and 10 gravel patches that do not include a redd of either species.
  - b. Collect control samples from the water column adjacent to each redd to establish background eDNA levels.
  - c. Analyze the samples using the developed molecular assays for Coho salmon (phase I) and Chinook salmon (Laramie and others, 2015).
  - d. Evaluate whether samples collected from Chinook and Coho redds have significantly higher levels of eDNA of the respective species than background levels (that is, from gravel, water column).
  - e. Evaluate whether samples collected from the interstitial spaces of gravel patches that are not redds are similar to background eDNA levels.

The Sandy River is a large tributary of the Columbia River (fig. 1). The Sandy River meets the Columbia River approximately 23 km upstream of Portland, Oregon. The Sandy River Basin provides overlapping spawning habitat for both Chinook and Coho salmon.

Samples provided by Portland Water Bureau for analysis were collected from the Bull Run River, Sixes Creek, Still Creek, Arrah Wanna Side Channel, and Side Channel 18 (table 1).



**Figure 1.** Map showing Sandy River Basin in northwestern Oregon. (Map provided by Burke Strobel, Portland Water Bureau on 23 February 2015.) Approximate location of study area: 45°24'09.94"N, 122°01'29.40"W.

**Table 1.** Environmental DNA (eDNA) samples with locations and date s.

[**Number of samples:** Each site consisted of seven samples, including three redd/gravel replicates, three water column replicates, and one distilled water control]

Sample location	Date sample collected	Number of sites	Number of samples
Bull Run River	11/14/2013	10	70
Bull Run River	11/26/2013	10	70
Sixes Creek	12/18/2013	4	28
Side Channel 18	12/16/2013	5	35
Arrah Wanna Side Channel	12/16/2013	1	7
Still Creek	01/15/2013	3	21
TOTALS		33	231

## Methods

Water samples were collected by Burke Strobel, Portland Water Bureau, using sterile syringes, and 15 mL of the sample were added to a 50 mL Falcon® tube containing 1.5 mL sodium acetate and 33 mL 200-proof EtOH. Falcon® tubes were chilled in a cooler in the field and placed in a refrigerator within 6 hours of sample collection. Samples were shipped to the U.S. Geological Survey (USGS) Snake River Field Station on January 30, 2014. Samples were sealed in plastic bags and surrounded with ice. Samples arrived in good condition—cool, no leakage. Samples were stored at -20 °C in a refrigerator until DNA extraction from March 24 to June 11, 2014.

DNA was extracted from samples using methods described in Ficetola and others (2008). Samples were first centrifuged at 5,500 g for 35 minutes at 6 °C. The supernatant was discarded. The remaining pellet was then subjected to DNA extraction using a Qiagen DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany), following the manufacturers protocol, with the only exception being that the final elution was done using 100 µL AE buffer to further concentrate DNA. To reduce the effects of inhibitors that may be present in stream water, all samples were purified by post-extraction spin-column purification using OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Irvine, California; Mckee and others, 2015). Purified DNA was stored in 0.5 mL cryo-vials at -20 °C.

We used an existing Chinook molecular assay (Laramie and others, 2015) for analysis of Chinook eDNA. The Coho assay was designed by Marshal Hoy (USGS). Molecular probes for both species were labeled with 6FAM at the 5' end and a minor groove binding non-fluorescent quencher (MGB-NFQ; Integrated DNA Technologies, Inc., Coralville, Iowa) at the 3' end. Additional assay specifications are shown in table 2. Both assays were screened for specificity using DNA extracted from non-target tissue samples. None of the non-target DNA, in concentrations likely to be encountered in a natural system, was amplified using the Chinook and Coho molecular assays. DNA from tissue samples used to determine the specificity of the qPCR assays were extracted using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, GmbH, Hilden, Germany), following the manufacturer's protocol. Extracted DNA was stored in 0.5 mL cryo-vials at -20 °C.

**Table 2.** Specifications of molecular assays for Coho salmon (*O. kisutch*) and Chinook salmon (*O. tshawytscha*).

Target species	Region	Base pairs	F-primer	R-primer	Probe
<sup>1</sup> <i>O. kisutch</i> (Coho)	<sup>3</sup> CYTB	114	CCT TGG TGG CGG ATA TAC TTA TCT TA	GAA CTAG GAA GAT GGC GAA GTA GAT C	6FAM-TGG AAC ACC CAT TCA T-MGBNFQ
<sup>2</sup> <i>O. tshawytscha</i> (Chinook)	<sup>4</sup> COI	90	CTG GCA CMG GGT GAA CAG TCT ACC	AAT GAA GGG AGA AGA TCG TYA GAT CA	6FAM-CTC CTG CGT GGG CTA G MBGNFQ

<sup>1</sup>Molecular assay developed by Marshal Hoy, Research Scientist, U.S. Geological Survey.

<sup>2</sup>Molecular assay developed by Laramie and others (2015).

<sup>3</sup>CYTB, cytochrome b region of the mitochondrial genome.

<sup>4</sup>COI, cytochrome oxidase subunit I region of the mitochondrial genome.

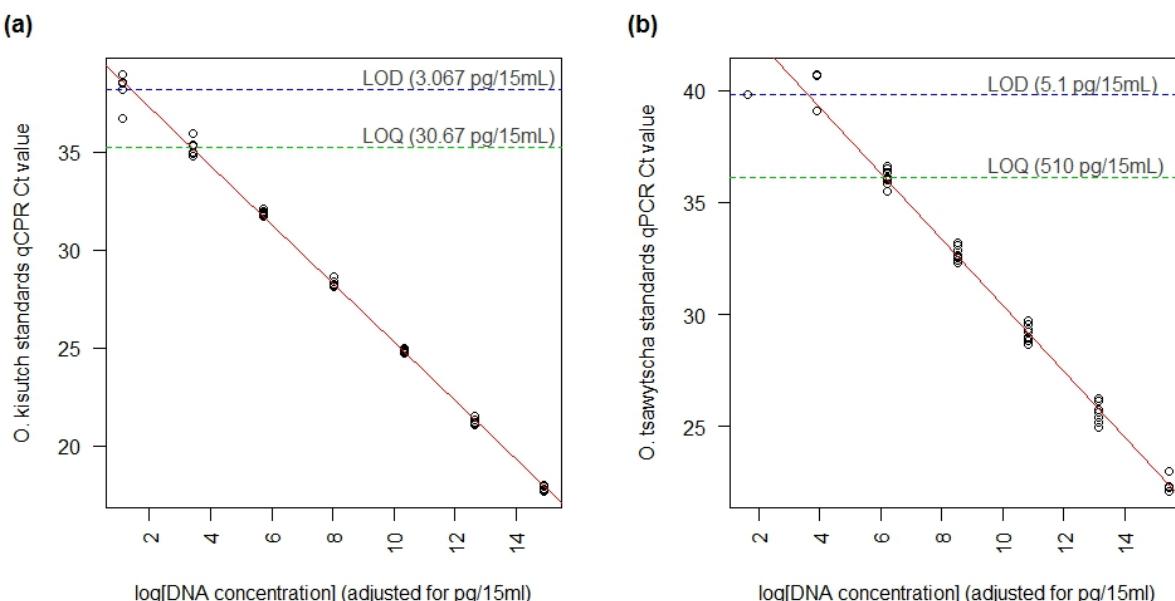
Reactions for qPCR analysis consisted of 15  $\mu$ L total volume, consisting of 0.06  $\mu$ L F-primer, 0.06  $\mu$ L R-primer, 0.03  $\mu$ L probe, 0.6  $\mu$ L AE buffer, 3.75  $\mu$ L DNase-free H<sub>2</sub>O, 7.5  $\mu$ L Quantitect Multiplex MasterMix, and 3  $\mu$ L sample DNA extract. All qPCR analysis was conducted on a Life Technologies 7300 Real-time qPCR System (Thermo Fisher Scientific, Inc., Waltham, Massachusetts). All qPCR reactions were singleplex.

Reactions using the Chinook assay used thermo-cycler conditions as follows: 2 minute warm up at 50 °C, 15 minute initial heat activation at 95 °C, followed by 50 cycles of 15 seconds denaturation at 94 °C and 60 seconds annealing/extension at 70 °C. Data were collected during the annealing/extension stage.

Reactions using the Coho assay utilized thermo-cycler conditions as follows: 2 minute warm up at 50 °C, 15-minute initial heat activation at 95 °C, followed by 50 cycles of 15 seconds denaturation at 94 °C and 60 seconds annealing/extension at 60 °C. Data were collected during the annealing/extension stage.

## Molecular Assay Performance

Standard curves for both Coho and Chinook salmon were created using eight-fold serial dilutions of DNA extracted from fin tissues of their respective species (fig. 2a, 2b).



**Figure 2.** Graphs showing (a) Coho salmon (*O. kisutch*) DNA Standards (10-fold dilutions), Slope: -3.452282, Intercept: 23.026682, R<sup>2</sup>: 0.997334, Limit of Detection (LOD), based on standard dilutions: 3.067x10<sup>-5</sup> ng/ $\mu$ L (3.067 pg/15 mL, mean Ct = 38.18962), Limit of Quantitation (LOQ), based on standard dilutions: 3.067e-004 ng/  $\mu$ L L (30.67 pg/15 mL, mean Ct = 35.26478) and (b) Chinook salmon (*O. tshawytscha*) DNA Standards (10-fold dilutions), Slope: -3.394368, Intercept: 28.193533, R<sup>2</sup>: 0.984555, LOD, based on standard dilutions: 5.1e-005 ng/ $\mu$ L (5.1 pg/15 mL, mean Ct = 39.7955), LOQ, based on standard dilutions: 0.0051 ng/ $\mu$ L (510 pg/15 mL, mean Ct = 36.12991).

## Data Analysis

An analysis of variance (ANOVA) was performed to compare mean eDNA concentrations (Coho and Chinook salmon) of each sample group; gravel, water, redds of both species, and three unknown redds. Tukey's Honestly Significant Difference test (multiple comparisons of means, 95-percent family-wise confidence level) was then performed to determine which group means were significantly different from each other. Means comparisons were plotted, along with 95-percent family-wise confidence intervals. All statistical analyses were performed using R Statistical software (R Core Team, 2013).

## Preliminary Results

**Analysis of negative controls.**—None of the negative controls amplified using Coho and Chinook salmon molecular assays, including (1) distilled water negative-controls collected at each sampling site, (2) DNA extraction laboratory negative-controls, and (3) qPCR non-template controls. These preliminary findings indicate that no within-site (among samples) or among-site contamination occurred in the field, nor did any contamination interfere with sample analysis.

**Sample analysis using the Coho assay.**—Analysis conducted using the Coho molecular assay detected eDNA concentrations ranging from 0 to 1,568.88 pg/15 mL, with a mean of 106.11 and median of 2.88 (fig. 3a). The sample type “*O. kisutch* redds” produced the highest mean concentration of Coho eDNA (494.65 pg/15 mL). Means for “*O. tshawytscha* redds,” “gravel,” and “water column” were 71.69 pg/15 mL, 103.95 pg/mL, and 10.1 pg/mL, respectively. ANOVA indicated a statistical difference between means of the sample group types (Chinook salmon redds, Coho salmon redds, gravel, water; table 3). A Tukey’s HSD means comparison indicated only three group mean comparisons were statistically significant; Coho salmon eDNA concentrations for Coho salmon redds differed from gravel, water column, and Chinook salmon redds (table 4). Figure 4a indicates the direction and difference between the various means comparisons performed along with 95 percent family-wise confidence levels.

**Figure 3.** Boxplots showing (a) distribution of Coho salmon (*O. kisutch*) eDNA concentrations at each sample site type, and (b) distribution of Chinook salmon (*O. tshawytscha*) eDNA concentrations at each sample site type. Thick horizontal lines represent sample medians, boxes represent quartiles, whiskers extend to minimum/maximum (excluding outliers), and circles represent outliers.

**Table 3.** Analysis of Variance (ANOVA) table for differences in Coho salmon (*O. kisutch*) eDNA among sample types.

	Degrees of freedom	Sum of squares	Mean Square	F value	Pr(>F)
Sample type	6	1847497	307916	8.1409	0.0000019 ***
Residuals	59	2231569	37823		
Significance codes:	0 ‘***’	0.001 ‘**’	0.01 ‘*’	0.05 ‘.’	0.1 ‘ ’ 1

Response: Site replicate mean [eDNA] (pg/15 mL)

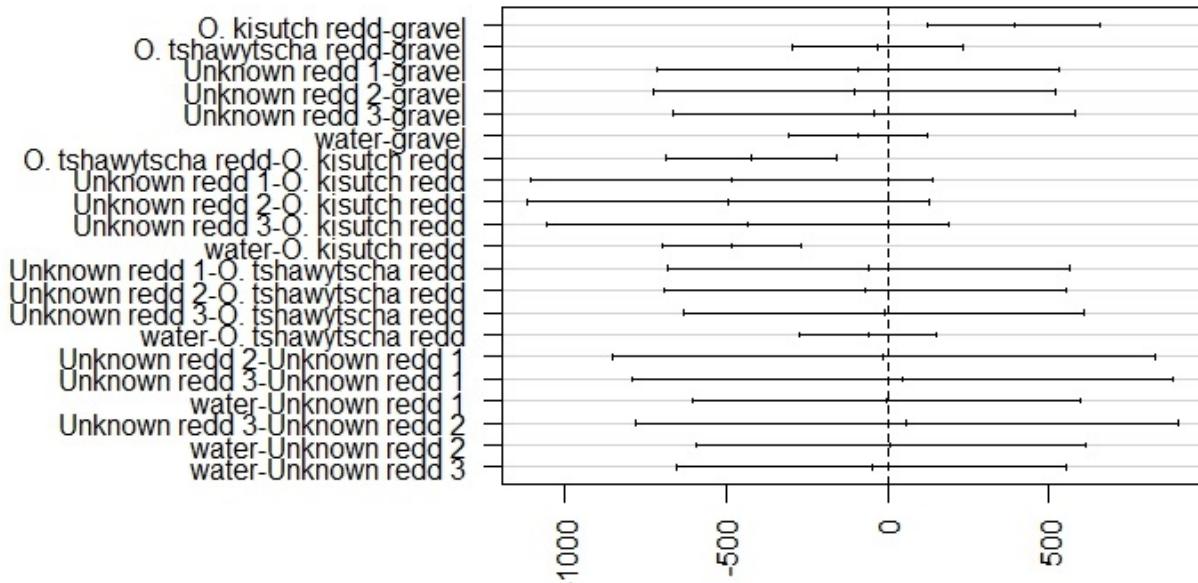
**Table 4.** Tukey multiple comparisons of means with 95 percent family-wise confidence level for Coho salmon (*O. kisutch*) eDNA among sample types.

Sample type	Difference	Lower bound	Upper bound	Adjusted p value
<i>O. kisutch</i> redd - gravel	390.692957	125.2171	656.1688	0.0006345
<i>O. tshawytscha</i> redd - <i>O. kisutch</i> redd	-422.953533	-688.4294	-157.4777	0.0001746
Water - <i>O. kisutch</i> redd	-484.550148	-698.8329	-270.2674	0.0000001

Fit: aov(formula = Site replicate mean [eDNA] (pg/15 mL) ~ Sample type, data = *O. kisutch* eDNA)

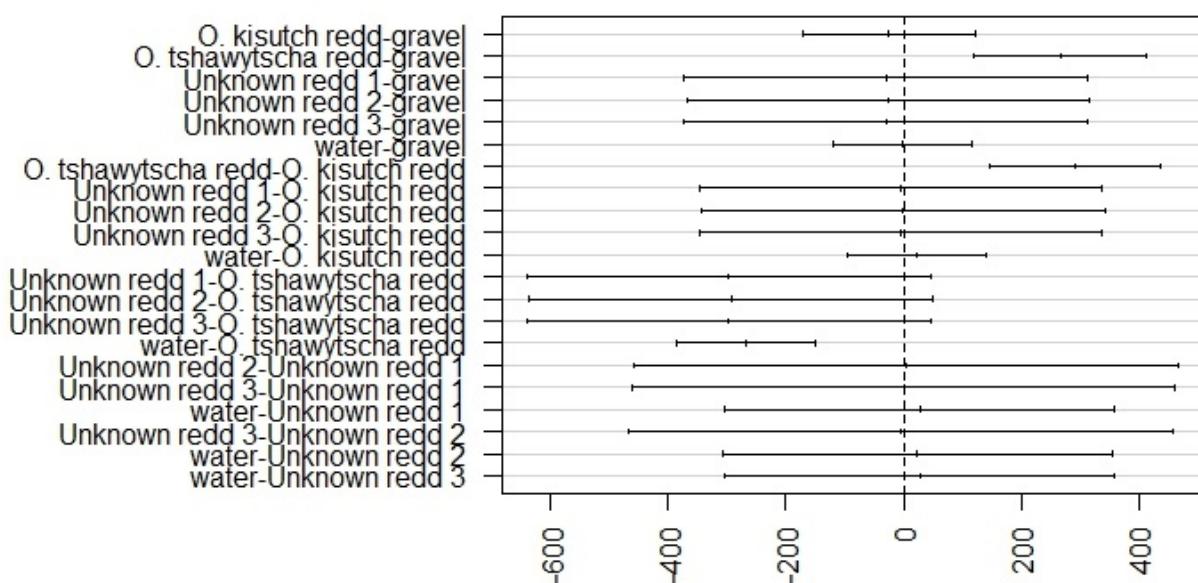
(a)

95% family-wise confidence level



(b)

95% family-wise confidence level



**Figure 4.** Graphs showing (a) comparisons of mean Coho salmon (*O. kisutch*) eDNA concentrations between all site types, (b) comparisons of mean Chinook salmon (*O. tshawytscha*) eDNA concentrations between all site types. X-axis represents mean-difference deviation from zero (pg/15 mL).

**Sample analysis using the Chinook assay.**—Analysis conducted using the Chinook molecular assay detected eDNA concentrations ranging from 0 to 870.69 pg/15 mL, with a mean of 63.98 and median of 4.57 (fig. 3b). The sample type “*O. tshawytscha* redds” produced the highest concentration of Chinook eDNA (296.64 pg/15 mL). Means for “*O. kisutch* redds,” “gravel,” and “water column” were 4.83 pg/15 mL, 29.89 pg/mL, and 27.43 pg/mL, respectively. ANOVA indicated a statistical difference between means of the sample group types (Chinook salmon redds, Coho salmon redds, gravel, water; table 5). A Tukey’s HSD means comparison indicated only three group mean-comparisons were statistically significant; Chinook salmon eDNA concentrations found at Chinook salmon redds differed from gravel, water column, and Coho salmon redds (table 6). Figure 4b indicates the direction and difference between the various means comparisons performed along with 95 percent family-wise confidence intervals.

**Table 5.** Analysis of Variance (ANOVA) table for differences in Chinook salmon (*O. tshawytscha*) environmental DNA (eDNA) among sample types.

	Degrees of freedom	Sum of squares	Mean Square	F value	Pr(>F)
Sample type	6	643758	107293	9.3578	3.318e-07 ***
Residuals	59	676473	11466		
Significance codes:	0 ‘***’	0.001 ‘**’	0.01 ‘*’	0.05 ‘.’	0.1 ‘ ’ 1

Response: Site replicate mean [eDNA] (pg/15 mL)

**Table 6.** Tukey multiple comparisons of means with 95 percent family-wise confidence level for Chinook salmon (*O. tshawytscha*) environmental DNA (eDNA) among sample types.

Sample type	Difference	Lower bound	Upper bound	Adjusted p-value
<i>O. tshawytscha</i> redd - gravel	266.7449	120.5793	412.91037	0.0000133
<i>O. tshawytscha</i> redd - <i>O. kisutch</i> redd	291.8099	145.6444	437.97538	0.0000018
Water - <i>O. tshawytscha</i> redd	-269.2079	-387.1876	-151.22823	0.0000001

Fit: aov(formula = Site replicate mean [eDNA] (pg/15mL)~ Sample type, data = O. tshawytscha eDNA)

**Sample analysis and assignment of three Unknown redds.**—Chinook salmon mean eDNA concentrations at Unknown redd 1, Unknown redd 2, and Unknown redd 3 were  $0.00 \pm 0.00\text{SE}$ ,  $4.14 \pm 4.14\text{SE}$ , and  $0.00 \pm 0.00\text{SE}$ , respectively. Coho salmon mean eDNA concentrations at Unknown redd 1, Unknown redd 2, and Unknown redd 3 were  $12.86 \pm 6.32\text{ SE}$ ,  $0.72 \pm 0.54\text{ SE}$ , and  $59.71 \pm 59.71\text{ SE}$ , respectively. The eDNA concentrations (for both species) recovered from these unknown redds was either zero or within the background levels of water or gravel. Therefore, none of the three unknown redds could confidently be assigned to either Chinook or Coho redds. However, Coho salmon eDNA concentrations were higher at Unknown redd 1 and Unknown redd 3, while Chinook salmon eDNA concentrations were only detected at Unknown redd 2.

*Note: Two sets of unknown redd sample vials (a set consists of three replicates of redd, three replicates of water, and one DI negative control) were labeled as “Unk 1.” It was not possible to determine which sample set was mislabeled, as both sets were otherwise identical. The vials were isolated and shipped in separate plastic bags, a process that allowed for discrimination between the two sample sets despite the identical labels. Therefore, one set of samples was arbitrarily converted in the laboratory to “Unk 2” prior to qPCR analysis. This change did not affect analysis or results.*

## Acknowledgments

Coho salmon molecular assay was developed by Marshal Hoy, Research Scientist, U.S. Geological Survey. We thank Boise State University for use of some equipment and laboratory space.

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**Appendix 1. Environmental DNA (eDNA) Concentrations for Coho Salmon (*O. kisutch*) and Chinook salmon (*O. tshawytscha*) from All Sites Sampled in the Sandy River Basin, northwestern Oregon, fall and winter 2013**

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
CK01RA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	1
CK01RB	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	2
CK01RC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	3
CK01WA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	1
CK01WB	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	2
CK01WC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	3
CK02RA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	1
CK02RB	11/14/2013	Bull Run River	O. kisutch CytB	41.1153	O. tshawytscha (Chinook)	Redd	2
CK02RC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	3
CK02WA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	1
CK02WB	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	2
CK02WC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	3
CK03RA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	1
CK03RB	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	2
CK03RC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	3
CK03WA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	1
CK03WB	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	2
CK03WC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	3
CK04RA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	1
CK04RB	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	2
CK04RC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	Redd	3
CK04WA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	1
CK04WB	11/14/2013	Bull Run River	O. kisutch CytB	0.2080	O. tshawytscha (Chinook)	water	2
CK04WC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	3

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
CK05RA	11/14/2013	Bull Run River	O. kisutch CytB	2.1067	O. tshawytscha (Chinook)	Redd	1
CK05RB	11/14/2013	Bull Run River	O. kisutch CytB	1.7400	O. tshawytscha (Chinook)	Redd	2
CK05RC	11/14/2013	Bull Run River	O. kisutch CytB	2.1300	O. tshawytscha (Chinook)	Redd	3
CK05WA	11/14/2013	Bull Run River	O. kisutch CytB	0.1987	O. tshawytscha (Chinook)	water	1
CK05WB	11/14/2013	Bull Run River	O. kisutch CytB	0.1847	O. tshawytscha (Chinook)	water	2
CK05WC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	3
CK06RA	11/14/2013	Bull Run River	O. kisutch CytB	1.6367	O. tshawytscha (Chinook)	Redd	1
CK06RB	11/14/2013	Bull Run River	O. kisutch CytB	2.2663	O. tshawytscha (Chinook)	Redd	2
CK06RC	11/14/2013	Bull Run River	O. kisutch CytB	0.4167	O. tshawytscha (Chinook)	Redd	3
CK06WA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	1
CK06WB	11/14/2013	Bull Run River	O. kisutch CytB	0.5763	O. tshawytscha (Chinook)	water	2
CK06WC	11/14/2013	Bull Run River	O. kisutch CytB	1.0133	O. tshawytscha (Chinook)	water	3
CK07RA	11/14/2013	Bull Run River	O. kisutch CytB	211.6590	O. tshawytscha (Chinook)	Redd	1
CK07RB	11/14/2013	Bull Run River	O. kisutch CytB	319.2550	O. tshawytscha (Chinook)	Redd	2
CK07RC	11/14/2013	Bull Run River	O. kisutch CytB	333.0243	O. tshawytscha (Chinook)	Redd	3
CK07WA	11/14/2013	Bull Run River	O. kisutch CytB	41.7900	O. tshawytscha (Chinook)	water	1
CK07WB	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	2
CK07WC	11/14/2013	Bull Run River	O. kisutch CytB	29.4000	O. tshawytscha (Chinook)	water	3
CK08RA	11/14/2013	Bull Run River	O. kisutch CytB	95.8007	O. tshawytscha (Chinook)	Redd	1
CK08RB	11/14/2013	Bull Run River	O. kisutch CytB	760.4840	O. tshawytscha (Chinook)	Redd	2
CK08RC	11/14/2013	Bull Run River	O. kisutch CytB	365.7090	O. tshawytscha (Chinook)	Redd	3
CK08WA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	1
CK08WB	11/14/2013	Bull Run River	O. kisutch CytB	10.7333	O. tshawytscha (Chinook)	water	2
CK08WC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	3
CK09RA	11/14/2013	Bull Run River	O. kisutch CytB	0.7730	O. tshawytscha (Chinook)	Redd	1
CK09RB	11/14/2013	Bull Run River	O. kisutch CytB	7.2533	O. tshawytscha (Chinook)	Redd	2
CK09RC	11/14/2013	Bull Run River	O. kisutch CytB	0.6067	O. tshawytscha (Chinook)	Redd	3
CK09WA	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	1
CK09WB	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	2
CK09WC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	3

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
CK10RA	11/14/2013	Bull Run River	O. kisutch CytB	0.5240	O. tshawytscha (Chinook)	Redd	1
CK10RB	11/14/2013	Bull Run River	O. kisutch CytB	1.0600	O. tshawytscha (Chinook)	Redd	2
CK10RC	11/14/2013	Bull Run River	O. kisutch CytB	3.2233	O. tshawytscha (Chinook)	Redd	3
CK10WA	11/14/2013	Bull Run River	O. kisutch CytB	0.7700	O. tshawytscha (Chinook)	water	1
CK10WB	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	2
CK10WC	11/14/2013	Bull Run River	O. kisutch CytB	0.0000	O. tshawytscha (Chinook)	water	3
CO01RA	12/18/2013	Sixes Creek	O. kisutch CytB	873.1010	O. kisutch (Coho)	Redd	1
CO01RB	12/18/2013	Sixes Creek	O. kisutch CytB	540.3803	O. kisutch (Coho)	Redd	2
CO01RC	12/18/2013	Sixes Creek	O. kisutch CytB	336.4547	O. kisutch (Coho)	Redd	3
CO01WA	12/18/2013	Sixes Creek	O. kisutch CytB	85.8000	O. kisutch (Coho)	water	1
CO01WB	12/18/2013	Sixes Creek	O. kisutch CytB	53.1010	O. kisutch (Coho)	water	2
CO01WC	12/18/2013	Sixes Creek	O. kisutch CytB	0.0000	O. kisutch (Coho)	water	3
CO02RA	12/18/2013	Sixes Creek	O. kisutch CytB	0.0000	O. kisutch (Coho)	Redd	1
CO02RB	12/18/2013	Sixes Creek	O. kisutch CytB	320.9270	O. kisutch (Coho)	Redd	2
CO02RC	12/18/2013	Sixes Creek	O. kisutch CytB	0.0000	O. kisutch (Coho)	Redd	3
CO02WA	12/18/2013	Sixes Creek	O. kisutch CytB	12.5667	O. kisutch (Coho)	water	1
CO02WB	12/18/2013	Sixes Creek	O. kisutch CytB	4.2000	O. kisutch (Coho)	water	2
CO02WC	12/18/2013	Sixes Creek	O. kisutch CytB	13.4000	O. kisutch (Coho)	water	3
CO03RA	12/16/2013	Side Channel 18	O. kisutch CytB	0.0000	O. kisutch (Coho)	Redd	1
CO03RB	12/16/2013	Side Channel 18	O. kisutch CytB	0.0000	O. kisutch (Coho)	Redd	2
CO03RC	12/16/2013	Side Channel 18	O. kisutch CytB	1022.4900	O. kisutch (Coho)	Redd	3
CO03WA	12/16/2013	Side Channel 18	O. kisutch CytB	2.3600	O. kisutch (Coho)	water	1
CO03WB	12/16/2013	Side Channel 18	O. kisutch CytB	3.0100	O. kisutch (Coho)	water	2
CO03WC	12/16/2013	Side Channel 18	O. kisutch CytB	5.3300	O. kisutch (Coho)	water	3
CO04RA	12/18/2013	Sixes Creek	O. kisutch CytB	154.4717	O. kisutch (Coho)	Redd	1
CO04RB	12/18/2013	Sixes Creek	O. kisutch CytB	32.7667	O. kisutch (Coho)	Redd	2
CO04RC	12/18/2013	Sixes Creek	O. kisutch CytB	10.8267	O. kisutch (Coho)	Redd	3
CO04WA	12/18/2013	Sixes Creek	O. kisutch CytB	1.2533	O. kisutch (Coho)	water	1
CO04WB	12/18/2013	Sixes Creek	O. kisutch CytB	3.3267	O. kisutch (Coho)	water	2
CO04WC	12/18/2013	Sixes Creek	O. kisutch CytB	2.2993	O. kisutch (Coho)	water	3

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
CO05RA	12/16/2013	Side Channel 18	O. kisutch CytB	1101.0497	O. kisutch (Coho)	Redd	1
CO05RB	12/16/2013	Side Channel 18	O. kisutch CytB	466.4827	O. kisutch (Coho)	Redd	2
CO05RC	12/16/2013	Side Channel 18	O. kisutch CytB	581.6717	O. kisutch (Coho)	Redd	3
CO05WA	12/16/2013	Side Channel 18	O. kisutch CytB	40.3530	O. kisutch (Coho)	water	1
CO05WB	12/16/2013	Side Channel 18	O. kisutch CytB	16.9333	O. kisutch (Coho)	water	2
CO05WC	12/16/2013	Side Channel 18	O. kisutch CytB	18.1000	O. kisutch (Coho)	water	3
CO06RA	12/16/2013	Side Channel 18	O. kisutch CytB	79.3717	O. kisutch (Coho)	Redd	1
CO06RB	12/16/2013	Side Channel 18	O. kisutch CytB	792.9467	O. kisutch (Coho)	Redd	2
CO06RC	12/16/2013	Side Channel 18	O. kisutch CytB	365.4017	O. kisutch (Coho)	Redd	3
CO06WA	12/16/2013	Side Channel 18	O. kisutch CytB	1.2700	O. kisutch (Coho)	water	1
CO06WB	12/16/2013	Side Channel 18	O. kisutch CytB	0.7017	O. kisutch (Coho)	water	2
CO06WC	12/16/2013	Side Channel 18	O. kisutch CytB	2.1533	O. kisutch (Coho)	water	3
CO07RA	12/16/2013	Arrah Wanna Side Channel	O. kisutch CytB	538.9067	O. kisutch (Coho)	Redd	1
CO07RB	12/16/2013	Arrah Wanna Side Channel	O. kisutch CytB	358.3360	O. kisutch (Coho)	Redd	2
CO07RC	12/16/2013	Arrah Wanna Side Channel	O. kisutch CytB	183.6263	O. kisutch (Coho)	Redd	3
CO07WA	12/16/2013	Arrah Wanna Side Channel	O. kisutch CytB	5.0467	O. kisutch (Coho)	water	1
CO07WB	12/16/2013	Arrah Wanna Side Channel	O. kisutch CytB	5.1600	O. kisutch (Coho)	water	2
CO07WC	12/16/2013	Arrah Wanna Side Channel	O. kisutch CytB	2.2967	O. kisutch (Coho)	water	3
CO08RA	12/16/2013	Side Channel 18	O. kisutch CytB	1989.5000	O. kisutch (Coho)	Redd	1
CO08RB	12/16/2013	Side Channel 18	O. kisutch CytB	70.2000	O. kisutch (Coho)	Redd	2
CO08RC	12/16/2013	Side Channel 18	O. kisutch CytB	3.7667	O. kisutch (Coho)	Redd	3
CO08WA	12/16/2013	Side Channel 18	O. kisutch CytB	1.2567	O. kisutch (Coho)	water	1
CO08WB	12/16/2013	Side Channel 18	O. kisutch CytB	1.2467	O. kisutch (Coho)	water	2
CO08WC	12/16/2013	Side Channel 18	O. kisutch CytB	0.0000	O. kisutch (Coho)	water	3
CO09RA	12/16/2013	Side Channel 18	O. kisutch CytB	223.5923	O. kisutch (Coho)	Redd	1
CO09RB	12/16/2013	Side Channel 18	O. kisutch CytB	22.4000	O. kisutch (Coho)	Redd	2
CO09RC	12/16/2013	Side Channel 18	O. kisutch CytB	64.0667	O. kisutch (Coho)	Redd	3
CO09WA	12/16/2013	Side Channel 18	O. kisutch CytB	1.4370	O. kisutch (Coho)	water	1

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
CO09WB	12/16/2013	Side Channel 18	O. kisutch CytB	2.3200	O. kisutch (Coho)	water	2
CO09WC	12/16/2013	Side Channel 18	O. kisutch CytB	2.5967	O. kisutch (Coho)	water	3
CO10RA	12/18/2013	Sixes Creek	O. kisutch CytB	32.3667	O. kisutch (Coho)	Redd	1
CO10RB	12/18/2013	Sixes Creek	O. kisutch CytB	8.8033	O. kisutch (Coho)	Redd	2
CO10RC	12/18/2013	Sixes Creek	O. kisutch CytB	4665.4833	O. kisutch (Coho)	Redd	3
CO10WA	12/18/2013	Sixes Creek	O. kisutch CytB	1.2310	O. kisutch (Coho)	water	1
CO10WB	12/18/2013	Sixes Creek	O. kisutch CytB	0.2007	O. kisutch (Coho)	water	2
CO10WC	12/18/2013	Sixes Creek	O. kisutch CytB	0.1843	O. kisutch (Coho)	water	3
GR01A	11/26/2013	Bull Run River	O. kisutch CytB	0.7667	None	Gravel	1
GR01B	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	Gravel	2
GR01C	11/26/2013	Bull Run River	O. kisutch CytB	4.9167	None	Gravel	3
GR01WA	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	1
GR01WB	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	2
GR01WC	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	3
GR02A	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	Gravel	1
GR02B	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	Gravel	2
GR02C	11/26/2013	Bull Run River	O. kisutch CytB	110.5487	None	Gravel	3
GR02WA	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	1
GR02WB	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	2
GR02WC	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	3
GR03A	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	Gravel	1
GR03B	11/26/2013	Bull Run River	O. kisutch CytB	0.7647	None	Gravel	2
GR03C	11/26/2013	Bull Run River	O. kisutch CytB	0.4500	None	Gravel	3
GR03WA	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	1
GR03WB	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	2
GR03WC	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	3
GR04A	11/26/2013	Bull Run River	O. kisutch CytB	38.3617	None	Gravel	1
GR04B	11/26/2013	Bull Run River	O. kisutch CytB	102.1077	None	Gravel	2
GR04C	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	Gravel	3
GR04WA	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	1

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
GR04WB	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	2
GR04WC	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	3
GR05A	11/26/2013	Bull Run River	O. kisutch CytB	288.8107	None	Gravel	1
GR05B	11/26/2013	Bull Run River	O. kisutch CytB	254.0207	None	Gravel	2
GR05C	11/26/2013	Bull Run River	O. kisutch CytB	175.3717	None	Gravel	3
GR05WA	11/26/2013	Bull Run River	O. kisutch CytB	87.3133	None	water	1
GR05WB	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	2
GR05WC	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	3
GR06A	11/26/2013	Bull Run River	O. kisutch CytB	183.3780	None	Gravel	1
GR06B	11/26/2013	Bull Run River	O. kisutch CytB	113.1340	None	Gravel	2
GR06C	11/26/2013	Bull Run River	O. kisutch CytB	1151.8350	None	Gravel	3
GR06WA	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	1
GR06WB	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	2
GR06WC	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	3
GR07A	11/26/2013	Bull Run River	O. kisutch CytB	0.6267	None	Gravel	1
GR07B	11/26/2013	Bull Run River	O. kisutch CytB	0.0076	None	Gravel	2
GR07C	11/26/2013	Bull Run River	O. kisutch CytB	9.7167	None	Gravel	3
GR07WA	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	1
GR07WB	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	2
GR07WC	11/26/2013	Bull Run River	O. kisutch CytB	0.4267	None	water	3
GR08A	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	Gravel	1
GR08B	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	Gravel	2
GR08C	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	Gravel	3
GR08WA	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	1
GR08WB	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	2
GR08WC	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	3
GR09A	11/26/2013	Bull Run River	O. kisutch CytB	81.7003	None	Gravel	1
GR09B	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	Gravel	2
GR09C	11/26/2013	Bull Run River	O. kisutch CytB	583.7227	None	Gravel	3
GR09WA	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	1

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
GR09WB	11/26/2013	Bull Run River	O. kisutch CytB	203.8467	None	water	2
GR09WC	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	3
GR10A	11/26/2013	Bull Run River	O. kisutch CytB	16.7333	None	Gravel	1
GR10B	11/26/2013	Bull Run River	O. kisutch CytB	0.0080	None	Gravel	2
GR10C	11/26/2013	Bull Run River	O. kisutch CytB	1.6200	None	Gravel	3
GR10WA	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	1
GR10WB	11/26/2013	Bull Run River	O. kisutch CytB	0.2557	None	water	2
GR10WC	11/26/2013	Bull Run River	O. kisutch CytB	0.0000	None	water	3
UNK01RA	1/15/2013	Still Creek	O. kisutch CytB	3.8813	Unknown	Redd	1
UNK01RB	1/15/2013	Still Creek	O. kisutch CytB	25.0667	Unknown	Redd	2
UNK01RC	1/15/2013	Still Creek	O. kisutch CytB	9.6367	Unknown	Redd	3
UNK01WA	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	water	1
UNK01WB	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	water	2
UNK01WC	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	water	3
UNK02RA	1/15/2013	Still Creek	O. kisutch CytB	1.7833	Unknown	Redd	1
UNK02RB	1/15/2013	Still Creek	O. kisutch CytB	0.3733	Unknown	Redd	2
UNK02RC	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	Redd	3
UNK02WA	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	water	1
UNK02WB	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	water	2
UNK02WC	1/15/2013	Still Creek	O. kisutch CytB	284.8343	Unknown	water	3
UNK03RA	1/15/2013	Still Creek	O. kisutch CytB	179.1353	Unknown	Redd	1
UNK03RB	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	Redd	2
UNK03RC	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	Redd	3
UNK03WA	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	water	1
UNK03WB	1/15/2013	Still Creek	O. kisutch CytB	0.0000	Unknown	water	2
UNK03WC	1/15/2013	Still Creek	O. kisutch CytB	48.8367	Unknown	water	3
CK01RA	11/14/2013	Bull Run River	O. tshawytscha COI	178.5853	O. tshawytscha (Chinook)	Redd	1
CK01RB	11/14/2013	Bull Run River	O. tshawytscha COI	260.4847	O. tshawytscha (Chinook)	Redd	2
CK01RC	11/14/2013	Bull Run River	O. tshawytscha COI	89.0983	O. tshawytscha (Chinook)	Redd	3
CK01WA	11/14/2013	Bull Run River	O. tshawytscha COI	87.6147	O. tshawytscha (Chinook)	water	1

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
CK01WB	11/14/2013	Bull Run River	O. tshawytscha COI	218.5640	O. tshawytscha (Chinook)	water	2
CK01WC	11/14/2013	Bull Run River	O. tshawytscha COI	16.1000	O. tshawytscha (Chinook)	water	3
CK02RA	11/14/2013	Bull Run River	O. tshawytscha COI	261.5873	O. tshawytscha (Chinook)	Redd	1
CK02RB	11/14/2013	Bull Run River	O. tshawytscha COI	166.3707	O. tshawytscha (Chinook)	Redd	2
CK02RC	11/14/2013	Bull Run River	O. tshawytscha COI	219.1917	O. tshawytscha (Chinook)	Redd	3
CK02WA	11/14/2013	Bull Run River	O. tshawytscha COI	78.9000	O. tshawytscha (Chinook)	water	1
CK02WB	11/14/2013	Bull Run River	O. tshawytscha COI	199.3390	O. tshawytscha (Chinook)	water	2
CK02WC	11/14/2013	Bull Run River	O. tshawytscha COI	378.6940	O. tshawytscha (Chinook)	water	3
CK03RA	11/14/2013	Bull Run River	O. tshawytscha COI	344.0073	O. tshawytscha (Chinook)	Redd	1
CK03RB	11/14/2013	Bull Run River	O. tshawytscha COI	335.5297	O. tshawytscha (Chinook)	Redd	2
CK03RC	11/14/2013	Bull Run River	O. tshawytscha COI	87.0577	O. tshawytscha (Chinook)	Redd	3
CK03WA	11/14/2013	Bull Run River	O. tshawytscha COI	13.7333	O. tshawytscha (Chinook)	water	1
CK03WB	11/14/2013	Bull Run River	O. tshawytscha COI	57.9100	O. tshawytscha (Chinook)	water	2
CK03WC	11/14/2013	Bull Run River	O. tshawytscha COI	35.1333	O. tshawytscha (Chinook)	water	3
CK04RA	11/14/2013	Bull Run River	O. tshawytscha COI	201.5137	O. tshawytscha (Chinook)	Redd	1
CK04RB	11/14/2013	Bull Run River	O. tshawytscha COI	259.8697	O. tshawytscha (Chinook)	Redd	2
CK04RC	11/14/2013	Bull Run River	O. tshawytscha COI	240.3683	O. tshawytscha (Chinook)	Redd	3
CK04WA	11/14/2013	Bull Run River	O. tshawytscha COI	121.2920	O. tshawytscha (Chinook)	water	1
CK04WB	11/14/2013	Bull Run River	O. tshawytscha COI	102.2447	O. tshawytscha (Chinook)	water	2
CK04WC	11/14/2013	Bull Run River	O. tshawytscha COI	47.1667	O. tshawytscha (Chinook)	water	3
CK05RA	11/14/2013	Bull Run River	O. tshawytscha COI	286.9430	O. tshawytscha (Chinook)	Redd	1
CK05RB	11/14/2013	Bull Run River	O. tshawytscha COI	63.1770	O. tshawytscha (Chinook)	Redd	2
CK05RC	11/14/2013	Bull Run River	O. tshawytscha COI	151.5153	O. tshawytscha (Chinook)	Redd	3
CK05WA	11/14/2013	Bull Run River	O. tshawytscha COI	30.2000	O. tshawytscha (Chinook)	water	1
CK05WB	11/14/2013	Bull Run River	O. tshawytscha COI	63.3197	O. tshawytscha (Chinook)	water	2
CK05WC	11/14/2013	Bull Run River	O. tshawytscha COI	20.4000	O. tshawytscha (Chinook)	water	3
CK06RA	11/14/2013	Bull Run River	O. tshawytscha COI	133.7667	O. tshawytscha (Chinook)	Redd	1
CK06RB	11/14/2013	Bull Run River	O. tshawytscha COI	280.2207	O. tshawytscha (Chinook)	Redd	2
CK06RC	11/14/2013	Bull Run River	O. tshawytscha COI	129.2073	O. tshawytscha (Chinook)	Redd	3
CK06WA	11/14/2013	Bull Run River	O. tshawytscha COI	90.7270	O. tshawytscha (Chinook)	water	1

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
CK06WB	11/14/2013	Bull Run River	O. tshawytscha COI	124.0723	O. tshawytscha (Chinook)	water	2
CK06WC	11/14/2013	Bull Run River	O. tshawytscha COI	113.9230	O. tshawytscha (Chinook)	water	3
CK07RA	11/14/2013	Bull Run River	O. tshawytscha COI	205.3013	O. tshawytscha (Chinook)	Redd	1
CK07RB	11/14/2013	Bull Run River	O. tshawytscha COI	0.0540	O. tshawytscha (Chinook)	Redd	2
CK07RC	11/14/2013	Bull Run River	O. tshawytscha COI	113.5967	O. tshawytscha (Chinook)	Redd	3
CK07WA	11/14/2013	Bull Run River	O. tshawytscha COI	27.9000	O. tshawytscha (Chinook)	water	1
CK07WB	11/14/2013	Bull Run River	O. tshawytscha COI	34.6667	O. tshawytscha (Chinook)	water	2
CK07WC	11/14/2013	Bull Run River	O. tshawytscha COI	116.7387	O. tshawytscha (Chinook)	water	3
CK08RA	11/14/2013	Bull Run River	O. tshawytscha COI	33.0369	O. tshawytscha (Chinook)	Redd	1
CK08RB	11/14/2013	Bull Run River	O. tshawytscha COI	109.1894	O. tshawytscha (Chinook)	Redd	2
CK08RC	11/14/2013	Bull Run River	O. tshawytscha COI	1818.2493	O. tshawytscha (Chinook)	Redd	3
CK08WA	11/14/2013	Bull Run River	O. tshawytscha COI	28.5985	O. tshawytscha (Chinook)	water	1
CK08WB	11/14/2013	Bull Run River	O. tshawytscha COI	36.2548	O. tshawytscha (Chinook)	water	2
CK08WC	11/14/2013	Bull Run River	O. tshawytscha COI	7.1027	O. tshawytscha (Chinook)	water	3
CK09RA	11/14/2013	Bull Run River	O. tshawytscha COI	806.5780	O. tshawytscha (Chinook)	Redd	1
CK09RB	11/14/2013	Bull Run River	O. tshawytscha COI	1310.4433	O. tshawytscha (Chinook)	Redd	2
CK09RC	11/14/2013	Bull Run River	O. tshawytscha COI	495.0417	O. tshawytscha (Chinook)	Redd	3
CK09WA	11/14/2013	Bull Run River	O. tshawytscha COI	53.3333	O. tshawytscha (Chinook)	water	1
CK09WB	11/14/2013	Bull Run River	O. tshawytscha COI	57.5633	O. tshawytscha (Chinook)	water	2
CK09WC	11/14/2013	Bull Run River	O. tshawytscha COI	45.1467	O. tshawytscha (Chinook)	water	3
CK10RA	11/14/2013	Bull Run River	O. tshawytscha COI	89.3580	O. tshawytscha (Chinook)	Redd	1
CK10RB	11/14/2013	Bull Run River	O. tshawytscha COI	152.3263	O. tshawytscha (Chinook)	Redd	2
CK10RC	11/14/2013	Bull Run River	O. tshawytscha COI	77.4720	O. tshawytscha (Chinook)	Redd	3
CK10WA	11/14/2013	Bull Run River	O. tshawytscha COI	76.1620	O. tshawytscha (Chinook)	water	1
CK10WB	11/14/2013	Bull Run River	O. tshawytscha COI	44.0000	O. tshawytscha (Chinook)	water	2
CK10WC	11/14/2013	Bull Run River	O. tshawytscha COI	20.1667	O. tshawytscha (Chinook)	water	3
CO01RA	12/18/2013	Sixes Creek	O. tshawytscha COI	3.9233	O. kisutch (Coho)	Redd	1
CO01RB	12/18/2013	Sixes Creek	O. tshawytscha COI	2.5767	O. kisutch (Coho)	Redd	2
CO01RC	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	3
CO01WA	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	1

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
CO01WB	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	2
CO01WC	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	3
CO02RA	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	1
CO02RB	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	2
CO02RC	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	3
CO02WA	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	1
CO02WB	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	2
CO02WC	12/18/2013	Sixes Creek	O. tshawytscha COI	12.1667	O. kisutch (Coho)	water	3
CO03RA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	1
CO03RB	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	2
CO03RC	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	3
CO03WA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	1
CO03WB	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	2
CO03WC	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	3
CO04RA	12/18/2013	Sixes Creek	O. tshawytscha COI	55.3100	O. kisutch (Coho)	Redd	1
CO04RB	12/18/2013	Sixes Creek	O. tshawytscha COI	78.6197	O. kisutch (Coho)	Redd	2
CO04RC	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	3
CO04WA	12/18/2013	Sixes Creek	O. tshawytscha COI	21.4333	O. kisutch (Coho)	water	1
CO04WB	12/18/2013	Sixes Creek	O. tshawytscha COI	5.9333	O. kisutch (Coho)	water	2
CO04WC	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	3
CO05RA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	1
CO05RB	12/16/2013	Side Channel 18	O. tshawytscha COI	1.6957	O. kisutch (Coho)	Redd	2
CO05RC	12/16/2013	Side Channel 18	O. tshawytscha COI	2.7200	O. kisutch (Coho)	Redd	3
CO05WA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	1
CO05WB	12/16/2013	Side Channel 18	O. tshawytscha COI	2.5333	O. kisutch (Coho)	water	2
CO05WC	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	3
CO06RA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	1
CO06RB	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	2
CO06RC	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	3
CO06WA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	1

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
CO06WB	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	2
CO06WC	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	3
CO07RA	12/16/2013	Arrah Wanna Side Channel	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	1
CO07RB	12/16/2013	Arrah Wanna Side Channel	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	2
CO07RC	12/16/2013	Arrah Wanna Side Channel	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	3
CO07WA	12/16/2013	Arrah Wanna Side Channel	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	1
CO07WB	12/16/2013	Arrah Wanna Side Channel	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	2
CO07WC	12/16/2013	Arrah Wanna Side Channel	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	3
CO08RA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	1
CO08RB	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	2
CO08RC	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	3
CO08WA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	1
CO08WB	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	2
CO08WC	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	3
CO09RA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	1
CO09RB	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	2
CO09RC	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	3
CO09WA	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	1
CO09WB	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	2
CO09WC	12/16/2013	Side Channel 18	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	3
CO10RA	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	1
CO10RB	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	2
CO10RC	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	Redd	3
CO10WA	12/18/2013	Sixes Creek	O. tshawytscha COI	14.9667	O. kisutch (Coho)	water	1
CO10WB	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	2
CO10WC	12/18/2013	Sixes Creek	O. tshawytscha COI	0.0000	O. kisutch (Coho)	water	3
GR01A	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	1
GR01B	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	2

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
GR01C	11/26/2013	Bull Run River	O. tshawytscha COI	36.0667	None	Gravel	3
GR01WA	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	1
GR01WB	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	2
GR01WC	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	3
GR02A	11/26/2013	Bull Run River	O. tshawytscha COI	6.3667	None	Gravel	1
GR02B	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	2
GR02C	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	3
GR02WA	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	1
GR02WB	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	2
GR02WC	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	3
GR03A	11/26/2013	Bull Run River	O. tshawytscha COI	239.5760	None	Gravel	1
GR03B	11/26/2013	Bull Run River	O. tshawytscha COI	168.0500	None	Gravel	2
GR03C	11/26/2013	Bull Run River	O. tshawytscha COI	6.7333	None	Gravel	3
GR03WA	11/26/2013	Bull Run River	O. tshawytscha COI	23.7667	None	water	1
GR03WB	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	2
GR03WC	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	3
GR04A	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	1
GR04B	11/26/2013	Bull Run River	O. tshawytscha COI	0.7200	None	Gravel	2
GR04C	11/26/2013	Bull Run River	O. tshawytscha COI	1.0067	None	Gravel	3
GR04WA	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	1
GR04WB	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	2
GR04WC	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	3
GR05A	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	1
GR05B	11/26/2013	Bull Run River	O. tshawytscha COI	0.2835	None	Gravel	2
GR05C	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	3
GR05WA	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	1
GR05WB	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	2
GR05WC	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	3
GR06A	11/26/2013	Bull Run River	O. tshawytscha COI	53.1492	None	Gravel	1
GR06B	11/26/2013	Bull Run River	O. tshawytscha COI	21.5795	None	Gravel	2

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
GR06C	11/26/2013	Bull Run River	O. tshawytscha COI	28.2565	None	Gravel	3
GR06WA	11/26/2013	Bull Run River	O. tshawytscha COI	50.9030	None	water	1
GR06WB	11/26/2013	Bull Run River	O. tshawytscha COI	42.5484	None	water	2
GR06WC	11/26/2013	Bull Run River	O. tshawytscha COI	0.2319	None	water	3
GR07A	11/26/2013	Bull Run River	O. tshawytscha COI	53.2333	None	Gravel	1
GR07B	11/26/2013	Bull Run River	O. tshawytscha COI	8.9333	None	Gravel	2
GR07C	11/26/2013	Bull Run River	O. tshawytscha COI	17.9000	None	Gravel	3
GR07WA	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	1
GR07WB	11/26/2013	Bull Run River	O. tshawytscha COI	81.7817	None	water	2
GR07WC	11/26/2013	Bull Run River	O. tshawytscha COI	20.8000	None	water	3
GR08A	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	1
GR08B	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	2
GR08C	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	3
GR08WA	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	1
GR08WB	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	2
GR08WC	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	3
GR09A	11/26/2013	Bull Run River	O. tshawytscha COI	3.9660	None	Gravel	1
GR09B	11/26/2013	Bull Run River	O. tshawytscha COI	24.6396	None	Gravel	2
GR09C	11/26/2013	Bull Run River	O. tshawytscha COI	220.4252	None	Gravel	3
GR09WA	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	1
GR09WB	11/26/2013	Bull Run River	O. tshawytscha COI	19.3195	None	water	2
GR09WC	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	3
GR10A	11/26/2013	Bull Run River	O. tshawytscha COI	0.3567	None	Gravel	1
GR10B	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	Gravel	2
GR10C	11/26/2013	Bull Run River	O. tshawytscha COI	5.5533	None	Gravel	3
GR10WA	11/26/2013	Bull Run River	O. tshawytscha COI	0.0000	None	water	1
GR10WB	11/26/2013	Bull Run River	O. tshawytscha COI	9.8000	None	water	2
GR10WC	11/26/2013	Bull Run River	O. tshawytscha COI	62.4333	None	water	3
UNK01RA	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	Redd	1
UNK01RB	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	Redd	2

Sample	Date	Location	Detector	eDNA (pg/15mL)	Species	Sample type	Replicate
UNK01RC	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	Redd	3
UNK01WA	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	water	1
UNK01WB	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	water	2
UNK01WC	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	water	3
UNK02RA	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	Redd	1
UNK02RB	1/15/2013	Still Creek	O. tshawytscha COI	12.4333	Unknown	Redd	2
UNK02RC	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	Redd	3
UNK02WA	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	water	1
UNK02WB	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	water	2
UNK02WC	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	water	3
UNK03RA	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	Redd	1
UNK03RB	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	Redd	2
UNK03RC	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	Redd	3
UNK03WA	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	water	1
UNK03WB	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	water	2
UNK03WC	1/15/2013	Still Creek	O. tshawytscha COI	0.0000	Unknown	water	3

**Appendix 2. Difference ( $\Delta$ ) Between Mean Environmental DNA (eDNA) Concentrations near the Substrate and in the Water Column (for *O. kisutch* and *O. tshawytscha*) at Each Site in the Sandy River Basin, northwestern Oregon, fall and winter 2013**

Site	Substrate eDNA Coho pg/15mL	Water eDNA Coho pg/15mL	$\Delta$ eDNA Coho	Substrate eDNA Chinook pg/15mL	Water eDNA Chinook pg/15mL	$\Delta$ eDNA Chinook	Species	Date	Stream
CO01	583.3120	46.3003	537.0117	2.1667	0.0000	2.1667	Coho	12/18/2013	Sixes Creek
CO02	106.9757	10.0556	96.9201	0.0000	4.0556	-4.0556	Coho	12/18/2013	Sixes Creek
CO03	340.8300	3.5667	337.2633	0.0000	0.0000	0.0000	Coho	12/16/2013	Side Channel 18
CO04	66.0217	2.2931	63.7286	44.6432	9.1222	35.5210	Coho	12/18/2013	Sixes Creek
CO05	716.4013	25.1288	691.2726	1.4719	0.8444	0.6274	Coho	12/16/2013	Side Channel 18
CO06	412.5733	1.3750	411.1983	0.0000	0.0000	0.0000	Coho	12/16/2013	Side Channel 18
CO07	360.2897	4.1678	356.1219	0.0000	0.0000	0.0000	Coho	12/16/2013	Arrah Wanna Side Channel
CO08	687.8222	0.8344	686.9878	0.0000	0.0000	0.0000	Coho	12/16/2013	Side Channel 18
CO09	103.3530	2.1179	101.2351	0.0000	0.0000	0.0000	Coho	12/16/2013	Side Channel 18
CO10	1,568.8844	0.5387	1,568.3458	0.0000	4.9889	-4.9889	Coho	12/18/2013	Sixes Creek
CK01	0.0000	0.0000	0.0000	176.0561	107.4262	68.6299	Chinook	11/14/2013	Bull Run River
CK02	13.7051	0.0000	13.7051	215.7166	218.9777	-3.2611	Chinook	11/14/2013	Bull Run River
CK03	0.0000	0.0000	0.0000	255.5316	35.5922	219.9393	Chinook	11/14/2013	Bull Run River
CK04	0.0000	0.0693	-0.0693	233.9172	90.2344	143.6828	Chinook	11/14/2013	Bull Run River
CK05	1.9922	0.1278	1.8644	167.2118	37.9732	129.2386	Chinook	11/14/2013	Bull Run River
CK06	1.4399	0.5299	0.9100	181.0649	109.5741	71.4908	Chinook	11/14/2013	Bull Run River
CK07	287.9794	23.7300	264.2494	106.3173	59.7684	46.5489	Chinook	11/14/2013	Bull Run River
CK08	407.3312	3.5778	403.7534	653.4919	23.9853	629.5066	Chinook	11/14/2013	Bull Run River
CK09	2.8777	0.0000	2.8777	870.6877	52.0144	818.6732	Chinook	11/14/2013	Bull Run River
CK10	1.6024	0.2567	1.3458	106.3854	46.7762	59.6092	Chinook	11/14/2013	Bull Run River
GR01	1.8944	0.0000	1.8944	12.0222	0.0000	12.0222	None	11/26/2013	Bull Run River

Site	Substrate eDNA Coho pg/15mL	Water eDNA Coho pg/15mL	Δ eDNA Coho	Substrate eDNA Chinook pg/15mL	Water eDNA Chinook pg/15mL	Δ eDNA Chinook	Species	Date	Stream
GR02	36.8496	0.0000	36.8496	2.1222	0.0000	2.1222	None	11/26/2013	Bull Run River
GR03	0.4049	0.0000	0.4049	138.1198	7.9222	130.1976	None	11/26/2013	Bull Run River
GR04	46.8231	0.0000	46.8231	0.5756	0.0000	0.5756	None	11/26/2013	Bull Run River
GR05	239.4010	29.1044	210.2966	0.0945	0.0000	0.0945	None	11/26/2013	Bull Run River
GR06	482.7823	0.0000	482.7823	34.3284	31.2278	3.1006	None	11/26/2013	Bull Run River
GR07	3.4503	0.1422	3.3081	26.6889	34.1939	-7.5050	None	11/26/2013	Bull Run River
GR08	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	None	11/26/2013	Bull Run River
GR09	221.8077	67.9489	153.8588	83.0103	6.4398	76.5704	None	11/26/2013	Bull Run River
GR10	6.1205	0.0852	6.0352	1.9700	24.0778	-22.1078	None	11/26/2013	Bull Run River
UNK01	12.8616	0.0000	12.8616	0.0000	0.0000	0.0000	Unknown	1/15/2013	Still Creek
UNK02	0.7189	94.9448	-94.2259	4.1444	0.0000	4.1444	Unknown	1/15/2013	Still Creek
UNK03	59.7118	16.2789	43.4329	0.0000	0.0000	0.0000	Unknown	1/15/2013	Still Creek



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