DISTRIBUTION OF CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) IN UPPER-COLUMBIA RIVER SUB-BASINS FROM ENVIRONMENTAL DNA ANALYSIS

by

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DEDICATION

For my parents.

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Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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ABSTRACT

Determining accurate species distribution is crucial to conservation and management strategies for imperiled species, but challenging for small populations that are approaching extinction or being reestablished. We evaluated the efficacy of environmental DNA (eDNA) analysis for improving detection and thus known distribution of Chinook salmon in the Methow and Okanogan Sub-basins of the Upper-Columbia River, Washington, USA. We developed an assay to target a 90 base pair sequence of Chinook DNA and used quantitative polymerase chain reaction (qPCR) to quantify the amount of Chinook eDNA in 1-L water samples collected at 48 sites in the sub-basins. We collected samples once during high flows in June and again during low flows in August 2012. Results from eDNA surveys were compared to the current known distribution of Chinook. Using eDNA methods, the probability of detecting Chinook given that they were present was 0.83. Detection probability was lower ($p = 0.69$) in June during high flows and at the beginning of spring-Chinook migration than during base flows in August ($p = 0.98$). Based on our triplicate sampling, we had a false-negative rate of 0.07, suggesting that fewer replicates could be collected at a site while maintaining reasonable detection. Of sites that tested positive during both sampling events, there was a higher mean concentration of eDNA in August than in June, probably because of reduced discharge, more fish, or both. As expected, eDNA concentration increased from upstream to downstream, but only in one tributary and this pattern varied considerably among streams suggesting that other factors influence the spatial pattern of eDNA

concentrations. For example, highest eDNA concentrations were found at sites with water temperatures centered around the optimal rearing temperature for Chinook and decreased rapidly around the approximate lethal temperature for the species. These results demonstrate the potential effectiveness of eDNA detection methods for determining landscape-level distribution of anadramous salmonids in large river systems.

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LIST OF ABBREVIATIONS

AKDFG	Alaska Department of Fish and Game
AFTC	Abernathy Fish Technology Center
BPA	Bonneville Power Administration
BSU	Boise State University
CCT	Colville Confederate Tribes
CCT F&W	Colville Confederated Tribes - Fish and Wildlife Department
CJHP	Chief Joseph Hatchery Program
CRITFC	Columbia River Inter-tribal Fish Commission
eDNA	Environmental DNA
ENFH	Entiat National Fish Hatchery
EtOH	Ethanol, molecular grade (200 proof)
ESA	Endangered Species Act
ESU	Evolutionarily Significant Unit
GC	Graduate College
GNLCC	Great Northern Landscape Conservation Cooperative
IDFG	Idaho Department of Fish and Game

LNFH	Leavenworth National Fish Hatchery
MDNR	Minnesota Department of Natural Resources
NCBI	National Center for Biotechnology Information
TDC	Thesis and Dissertation Coordinator
UCSRB	Upper Columbia Salmon Recovery Board
UI	University of Idaho
USGS	United States Geological Survey
USWFS	United States Fish and Wildlife Service
WDFW	Washington Department of Fish and Wildlife
WNFH	Winthrop National Fish Hatchery

CHAPTER ONE: STUDY BACKGROUND

The two primary goals of this study were to (1) test the effectiveness of environmental DNA (eDNA) detection methods to determine the distribution of Chinook salmon (*Oncorhynchus tshawytscha*) in the Methow and the Okanogan Sub-basins, two large tributaries of the Upper-Columbia River, and (2) to provide baseline data for an eDNA monitoring program that could be used to track changes in Chinook distribution throughout the Okanogan Sub-basin following a proposed re-introduction of an experimental population of spring-Chinook by the Colville Confederated Tribes under Section 10(j) of the Endangered Species Act (ESA) (FR 76:42658 2011).

To my knowledge, this is a novel application of eDNA methods to detect anadromous salmonids in large, western watersheds

Study Area

The Methow and Okanogan Sub-basins encompass a combined 16,000 km^2 of north-central Washington State, USA and southern British Columbia, Canada, both draining into the Upper-Columbia River just downstream on Chief Joseph Dam, the upstream terminus for anadromous fish migration (Figure 1.1).

Figure 1.1 Study area - Methow and Okanogan Sub-basins in North-central Washington State, USA and Southern British Columbia, Canada.

During our sampling period, stream flow at the lowest site in the Methow Sub-basin, near the town of Pateros, ranged from 187.4 cubic meters per second (m^3/s) on 22 June 2012 to 23.1 m^3/s on 13 August 2012. During the same time period, stream flow at the lowest site on the Twisp River, a typical, large tributary in the Methow Sub-basin, ranged from 34.2 m^3/s to 3.6 m^3/s (Pictures 1.1 and 1.2, respectively).

Picture 1.1 Twisp River (Methow Sub-basin – WA, USA) high flow (June) 2012

Picture 1.2 Twisp River (Methow Sub-basin – WA, USA) low flow (August) 2012

Again during the same time period, stream flow at Nine Mile Creek, a small tributary in the Okanogan Sub-basin, ranged from 0.03 m^3/s to 0.009 m^3/s (Pictures 1.3 and 1.4)

Picture 1.3 Nine Mile Creek (Okanogan Sub-basin – WA, USA) high flow (June) 2012

Picture 1.4 Nine Mile Creek (Okanogan Sub-basin – WA, USA) low flow (August) 2012

Throughout both Sub-basins, stream flows were generally 10 times higher during spring run-off in June than later in August, when flows were approaching base-flow.

Study Species

Two life-history types of Chinook salmon were historically present in the Upper-Columbia River basin, a stream-type and an ocean-type (Healey 1991, Waples *et al.* 2004). Ocean-type Chinook adults migrate to freshwater during the summer and fall, spawning primarily in mainstem rivers. Stream-type Chinook migrate upstream during peak spring flows, which allow access to preferred spawning habitat in higher headwater tributaries (Healey 1991). Spawning for both life-history types takes place in late-summer and fall, but in different habitats (mainstem versus headwaters), resulting in near-complete reproductive isolation (Waples *et al.* 2004, Beacham *et al.* 2006, Narum *et al.* 2007). Upon emergence, juveniles of ocean-type Chinook migrate to the ocean during their first spring, as sub-yearlings, while stream-type juveniles remain in freshwater until their second spring before migrating to the ocean as yearlings (Healey 1991). Stream- and ocean-type Chinook will hereafter be referred to as spring- and fall-Chinook, respectively, which denotes timing of adult, upstream migration, and are the more commonly used terms. The Columbia River drainage once supported some of the largest runs of Chinook salmon known (Chapman 1986, Utter *et al.* 1989). Spring-Chinook of the Upper-Columbia River Evolutionarily Significant Unit (ESU) are now among the most imperiled North American salmon and are currently listed as Endangered under the Endangered Species Act (ESA) (FR 64:41839, 1999). The Methow Sub-basin still contains both fall- and spring-Chinook. The Okanogan Sub-basin currently contains only

fall-Chinook, while spring-Chinook were extirpated in the 1930's (FR 76:42658 2011). In 2012, 52,846 Chinook were counted as they migrated from the ocean, upstream passed Wells Dam, destined for the Methow and Okanogan Sub-basins (DeHart 2013).

Environmental DNA

Environmental DNA, or eDNA, is an emerging, genetic method used to detect aquatic species in a survey area by collecting, concentrating, and amplifying exogenous and persistent DNA from the environment. The source of eDNA is not completely understood, but likely comes from tissue and cells sloughed during excretion, molting, reproduction, injury, or death. Thus, eDNA may be in dissolved or intra-cellular form, and may be intact or fragmented, depending on processes of decomposition and degradation. Environmental DNA is known to persist in aquatic environments for up to approximately 3 weeks, depending on environmental conditions (Dejean *et al.* 2011, Thomsen *et al.* 2012a, Pilliod *et al.* 2013) and thus it provides a useful measure of species presence in biologically relevant time scales. Aquatic eDNA has been successfully used to detect species from water samples ranging from 15 mL to 5 L (Ficetola *et al.* 2008, Goldberg *et al.* 2011, Jerde *et al.* 2011).

Only recently have studies began examining the effectiveness of eDNA detection methods. The seminal study evaluated eDNA as a method for detecting invasive Bullfrogs (*Lithobates catesbeianus*) in freshwater ponds in France (Ficetola *et al.* 2008). The first application of eDNA methods in flowing (lotic) waters was to detect invasive Big headed carp (*Hypophthichthys nobilis*) and Silver carp (*Hypophthichthys molitrix*) in freshwater canals (Jerde *et al.* 2011). Since then, eDNA has effectively been used to detect Idaho giant salamanders (*Dicamptodon aterrimus*) and Rocky Mountain tailed

frogs (*Ascaphus montanus*) in small, high-gradient streams (Goldberg *et al.* 2011, Pilliod *et al.* 2013) and a number of additional species in a variety of habitats (Table 1.1).

Table 1.1 Studies using eDNA with species detected

Habitat	Species detected	Author
Aquariums, ponds	American bullfrog (*Lithobates catesbeianus*)	Ficetola *et al.* 2008
Large river/canal complex	Big headed carp (*Hypophthichthys nobilis*) and Silver carp (*Hypophthichthys molitrix*)	Jerde *et al.* 2011
Small, high-gradient streams	Idaho giant salamanders (*Dicamptodon aterrimus*) and Rocky Mountain tailed frogs (*Ascaphus montanus*)	Goldberg *et al.* 2011, Pilliod *et al.* 2013
Ponds, lakes, streams	Common spadefoot toad (*Pelobates fuscus*), Great crested newt (*Triturus cristatus*), European weather loach (*Misgurnus fossilis*), Eurasean otter (*lutra lutra*), White-faces darter (*Leucorrhinia pectoralis*), Tadpole shrimp (*Lepidurus apus*)	Thomsen *et al.* 2012a
Ocean	Pleuronectidae (*Pleuronectes platessa, Limanda limanda, Platicthys flesus*), Zoarcidae (*Zoarces viviparus*), Labridae (*Ctenolabrus rupestris*), Trachinidae (*Trachinus draco*), Anguillidae (*Anguilla Anguilla*), Salmonidae (*Salmo trutta*), Gadidae (*Gadus morhua*), Gasterosteidae (*Gasterosteus aculeatus*, *Spinachia spinachia*), Syngnathidae (*Syngnathus acus*), Clupeidae (*Sardina pilchardus*, *Clupea harengus*), Cottidae (*Myoxocephalus scorpius*), Gaviidae (*Gavia stellate*), Columbidae (*Columba livia*), Anatidae (Cygnus olor), Phalacrocoracidae	Thomsen *et al.* 2012b

	(*Phalacrocorax carbo*)	
Ponds, beakers	Sturgeon (*Acipenser baerii*), American bullfrog (*Lithobates catesbeianus*)	Dejean *et al.* 2011, Dejean *et al.* 2012
Aquariums, ponds, freshwater streams	Common carp (*Cyprinus carpio*)	Takahara *et al.* 2012
Ponds	Bluegill (*Lepomis macrochirus*)	Takahara *et al.* 2013
Streams	Brook trout (*Salvelinus fontinalis*), bull trout (*S. confluentus*)	Wilcox *et al.* 2013
Aquariums, river	Cyprinidae (*Nipponocypris temminckii*), Adrianichthyidae (*Oryzias latipes*), Centrarchidae (*Lepomis macrochirus*), Odontobutidae (*Odontobutis obscura*), Bagridae (*Pelteobagrus nudiceps*)	Minamoto *et al.* 2012

Only two studies have demonstrated the use of eDNA to detect salmonids, the first was a study that used deep sequencing to assess biodiversity of marine environments (Thomsen *et al.* 2012b), and the second, an examination of the factors influencing specificity and sensitivity of molecular assays (Wilcox *et al.* 2013).

Salmonid detection using eDNA has not yet been applied on a landscape level to determine species distribution throughout large basins and despite the advances that have been made in this field, to my knowledge to date, few fisheries management programs are taking advantage of this sensitive detection method.

Environmental DNA as a Potential Monitoring Tool for Chinook Salmon

The Colville Confederated Tribes Fish and Wildlife Department (CCT F&W) has proposed to re-establish spring-Chinook in the Okanogan Sub-basin beginning with the reintroduction of an experimental population under section 10(j) of the Endangered Species Act (ESA) (FR 76:42658 2011). The CCT F&W was also interested in implementing a monitoring program that utilized eDNA detection methods to determine, and track changes in, the distribution of fall- and spring-Chinook in the Okanogan following the 10(j) reintroduction. To accomplish this goal, I first developed an eDNA assay for the species using qPCR. Genetic differences between life-history types were insufficient to differentiate spring versus fall-Chinook with the chosen molecular marker, and thus my analyses were limited to the species-level (but see discussion in Chapter 2). I then evaluated the effectiveness of eDNA methods for determining the distribution of Chinook in the Methow Sub-basin, where both fall- and spring-Chinook are still fairly abundant and also in the Okanogan Sub-basin (Appendix A) where currently only fall-Chinook are present. These initial surveys in the Okanogan in 2012 will establish a baseline distribution of Chinook throughout the Okanogan (Appendix B), prior to the proposed reintroduction of spring-Chinook. Future eDNA monitoring in this Sub-basin would allow fisheries managers to track changes in Chinook distribution.

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CHAPTER TWO: CHARACTERIZING THE DISTRIBUTION OF AN ENDANGERED SALMONID USING ENVIRONMENTAL DNA ANALYSIS

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Title Characterizing the distribution of an endangered salmonid using environmental DNA analysis

Abstract Determining accurate species distribution is crucial to conservation and management strategies for imperiled species, but challenging for small populations that are approaching extinction or being reestablished. We evaluated the efficacy of environmental DNA (eDNA) analysis for improving detection and thus known distribution of Chinook salmon in the Methow and Okanogan Sub-basins of the Upper-Columbia River, Washington, USA. We developed an assay to target a 90 base pair sequence of Chinook DNA and used quantitative polymerase chain reaction (qPCR) to quantify the amount of Chinook eDNA in 1-L water samples collected at 48 sites in the sub-basins. We collected samples once during high flows in June and again during low flows in August 2012. Results from eDNA surveys were compared to the current known

distribution of Chinook. Using eDNA methods, the probability of detecting Chinook given that they were present was 0.83. Detection probability was lower ($p = 0.69$) in June during high flows and at the beginning of spring-Chinook migration than during base flows in August ($p = 0.98$). Based on our triplicate sampling, we had a false-negative rate of 0.07, suggesting that fewer replicates could be collected at a site while maintaining reasonable detection. Of sites that tested positive during both sampling events, there was a higher mean concentration of eDNA in August than in June, probably because of reduced discharge, more fish, or both. As expected, eDNA concentration increased from upstream to downstream, but only in one tributary and this pattern varied considerably among streams, suggesting that other factors influence the spatial pattern of eDNA concentrations. For example, highest eDNA concentrations were found at sites with water temperatures centered around the optimal rearing temperature for Chinook and decreased rapidly around the approximate lethal temperature for the species. These results demonstrate the potential effectiveness of eDNA detection methods for determining landscape-level distribution of anadramous salmonids in large river systems.

Keywords Methow, Okanogan, Oncorhynchus tsawytscha, spring-Chinook

Introduction

Salmon populations once abundant throughout the Pacific Northwest have declined dramatically, due largely to hydropower development, habitat degradation, and overharvest (Mullan 1987, Nehlsen *et al.* 1991, FR 76:42658 2011). The Columbia River drainage once supported some of the largest runs of Chinook salmon (*Oncorhynchus tshawytscha*) known (Chapman 1986, Utter *et al.* 1989). Spring-Chinook of the Upper-Columbia River Evolutionarily Significant Unit (ESU) are now among the most imperiled North American salmon and are currently listed as Endangered under the Endangered Species Act (ESA) (FR 64:41839, 1999). Costly conservation efforts such as hatchery supplementation, habitat restoration, and harvest management have been implemented to conserve remaining populations (LCFRB 2010, GAO RCED-93-41 1993).

Accurate species distribution modeling and ability to rapidly track responses to management strategies is important for assessing the status and effectiveness of conservation efforts and forms the basis of good decision making (Hernandez *et al.* 2006, Stem *et al.* 2005). One challenge of determining or confirming the distribution of an aquatic species is low detection rates, especially for species that are cryptic, secretive, or occur at low densities.

An emerging method that may improve detection of aquatic species is environmental DNA (eDNA) analysis. This method determines presence of a species based on the collection, concentration, and amplification of their DNA from the environment. Environmental DNA is genetic material from sloughed tissue and cells of plants and animals produced during excretion, reproduction, injury, or death. The

dissolved or intracellular DNA can be intact or fragmented, depending on decomposition and degradation processes. Environmental DNA appears to persist in aquatic environments for up to approximately 3 weeks, depending on conditions (Dejean *et al.* 2011, Thomsen *et al.* 2012a, Pilliod *et al.* 2013a). Environmental DNA is obtained by collecting or filtering a sample of water (Ficetola *et al.* 2008, Goldberg *et al.* 2011, Jerde *et al.* 2011).

Recent studies have demonstrated that eDNA detection can be a reliable method for determining the distribution of various species of aquatic amphibians (Ficetola *et al.* 2008, Goldberg *et al.* 2011, Thomsen *et al.* 2012a, Pilliod *et al.* 2013a, Pilliod *et al* . 2013b, Pilliod *et al*. 2013c) and fish in freshwater ecosystems (Jerde *et al.* 2011, Dejean *et al.* 2011, Minamoto *et al.* 2012, Takahara *et al.* 2012, Thomsen *et al.* 2012a, Takahara *et al.* 2013) as well as in oceans (Thomsen *et al.* 2012b). Particularly when determining presence of rare or low-density species, eDNA detection methods have been shown to be more sensitive than traditional sampling methods, such as electrofishing or visual surveys, and therefore can be a powerful tool for conservation and natural resource managers (Jerde *et al.* 2011, Lodge *et al.* 2012, Jerde *et al.* 2013, Takahara *et al.* 2012, Pilliod *et al.* 2013a). Studies have also shown positive correlation between eDNA concentration and relative abundance of the target organism (Thompson *et al.* 2012a, Takahara *et al.* 2012, Pilliod *et al.* 2013a, Pilliod *et al.* 2013b). Less work has been conducted in lotic systems and it is still unclear the upstream inference of eDNA results (Pilliod *et al.* 2013b).

Despite the demonstrated effectiveness of eDNA detection methods, few fisheries management programs are currently taking advantage of this state-of-the-art tool for

determining the presence of sensitive, native species. This study was designed to test the effectiveness of eDNA detection methods for determining the distribution of threatened and endangered Chinook salmon populations in the Methow and Okanogan Sub-basins of the Upper-Columbia River region by comparing a distribution resulting from eDNA detection to the current, known distribution of the species.

Methods

Study Species

Interior Columbia River Chinook are comprised of two lineages, described as ocean- and stream-type, each with a different life-history strategy (Healey 1991, Waples *et al.* 2004). Ocean-type Chinook adults migrate to freshwater throughout summer and fall and spawn primarily in mainstem rivers. Stream-type Chinook migrate upstream during peak spring flows, which allow them to access to preferred spawning habitat in higher headwater tributaries. Spawning takes place in the late summer and fall for both strains, but in different habitats resulting in near-complete reproductive isolation (Waples *et al.* 2004, Beacham *et al.* 2006, Narum *et al.* 2007). Upon emergence, juveniles of ocean-type Chinook migrate to the ocean their first spring, as sub-yearlings, while stream-type juveniles remain in freshwater until their second spring before migrating to the ocean as yearlings (Healey 1991). Hereafter, we will refer to stream- and ocean-type Chinook by their more commonly used names: spring- and fall-Chinook, respectively.

Study Area - Methow Sub-Basin

The Methow Sub-basin in western Okanogan County, Washington USA drains 2,900 km^2, via the Methow, Chewuch, and Twisp Rivers before emptying into the

Columbia River near Pateros, Washington (Figure 2.1). The Methow contains both spring- and fall-Chinook (UCSRB 2007). We used existing spring-Chinook distribution maps (UCSRB 2007) to select sites (n=32) categorized *a priori* as (1) Chinook likely present (i.e., within the known distribution of Chinook, n=21), or (2) Chinook likely absent (i.e., outside of the known distribution of Chinook, n=11). Three sample sites of the latter category were physically inaccessible to Chinook (above barriers to anadromy) and served as stream negative-controls. All sites in the Methow Sub-basin were sampled twice, once during high, spring-flows from 22–27 June 2012, and again during reduced late-summer flows from 9–13 August (Figure 2.2). In general, stream flows were approximately 10X higher during spring run-off in June than later in August, as flows approached base-flow. During June sampling, flows ranged from 242 m^3/s in the mainstem Methow River to <1 m^3/s in small tributaries.

Okanogan Sub-Basin

The Okanogan Sub-basin is adjacent to and east of the Methow and spans the border between Washington, United States and British Columbia, Canada (Figure 2.1). The Okanogan Sub-basin is more than four times the size of the Methow, draining approximately 13,000 km^2. The Okanogan contains fall-Chinook, while spring-Chinook were extirpated by the 1930's (UCSRB 2007). Migrating spring-Chinook adults from nearby sub-basins may occasionally stray into the Okanogan, suggesting potential for presence of a very low-density population (J. Arterburn, CCT F&W biologist, personal communication). The Colville Confederated Tribes plan to re-establish spring-Chinook throughout much of their historic range in the Okanogan as an experimental population under Section 10(j) of the ESA (FR 76:42658 2011). The source stock for the Okanogan

reintroduction would initially come from the adjacent Methow Sub-basin. We selected eDNA sample sites in the Okanogan on the basis of high potential for re-colonization because of suitable habitat characteristics. These surveys also will serve as baseline-distribution (prior to the reintroduction of spring-Chinook to the Okanogan Sub-basin) that can then be used as part of a monitoring program to track changes in Chinook distribution following the reintroduction. We sampled 16 sites in the Okanogan Sub-basin, once during high, spring-flows from 18–21 June 2012, and again during reduced late-summer flows from 14–17 August (Figure 2.2). As in the Methow Sub-basin, stream flows in the Okanogan were approximately 10X higher during spring run-off in June than later in August, as flows approached base-flow. During June sampling, flows ranged from 390.7 m^3/s in the mainstem Okanogan River to 0.03 m^3/s in small tributaries.

In 2012, 52,846 Chinook were counted as they migrated from the ocean upstream passed Wells Dam, on their way to the Methow and Okanogan Sub-basins (DeHart 2013).

Field Methods

At each sample site, we filtered three 1-L stream water replicates and one 1-L negative control composed of distilled water. The negative control was used to detect any contamination between sites. Water was filtered through a Whatman Disposable Filter Funnel with 47 mm diameter, 0.45 μm pore size cellulose nitrate type WCN sterile filter membrane. The filter funnel was connected to Masterflex silicone tubing and then fed through a Masterflex L/S Econodrive peristaltic pump with Masterflex L/S standard pump head and powered by a portable 12 volt battery. We held the filter funnel just below the surface of the stream, facing upstream, into the current. The pump was

engaged until 1-L of stream water was collected. We collected all samples along the edge of the streams, so rarely was it necessary to wade into the stream for collection. However, care was also taken to ensure that samples were collected in locations with adequate downstream flow.

We removed the filter from the disposable funnel using forceps and then placed it into a sterile 2-ml cryogenic vial filled with 1.8 ml of 200-proof ethanol, for preservation. The forceps were sterilized between each sample by submersion in a solution of 50% household bleach (6% sodium hypochlorite) and 50% distilled water for 2 minutes. Forceps were then rinsed thoroughly with distilled water before using. We wore disposable nitrile examination gloves while filtering water and handling filter paper. Sample vials were stored in plastic cryo-vial storage boxes away from sunlight and stored at 4C until DNA could be extracted. Water temperature was collected at each site at the time of sampling using a thermometer.

Molecular Assay Design

Markers were developed for qPCR analysis rather than conventional PCR to reduce the rate of false negatives (Wilcox *et al*. 2013) and reduce potential for contamination that may result from handling of high-copy number PCR product. The Chinook qPCR assay targeted a 90 base-pair sequence of the *cytochrome oxidase c subunit I* (COI) region within the mitochondrial genome, a region that has been targeted and sequenced for a wide range of organisms for DNA barcoding (Hebert and Gregory 2005). A Taq-Man (Life Technologies, Co.) assay was used with a probe containing 6-FAM dye at the 5'- end and a minor groove binding non-florescent quencher (MGB-NFQ) at the -3' end. Primer Express 3.0 software (Applied Biosystems, Inc.) was used to

evaluate and select the target amplicon with F-primer: *5'- CTG GCA CMG GGT GAA CAG TCT ACC-3'*, R-primer: *5'-AAT GAA GGG AGA AGA TCG TYA GAT CA-3'* (Integrated DNA Technologies, Inc.), and probe: *6FAM-CTC CTG CGT GGG CTA G-MBG-NFQ*). A BLAST search was conducted to ensure specificity of the assay. The selected assay contains a minimum of 3 dissimilar bases between Chinook and closest relative Coho salmon (*O. kisutch)* (Healey 1991).

Target species (Chinook) fin clips were collected from the Columbia River region (n=20), as were fin clips from non-target species (*Oncorhynchus mykiss*, *O. clarki*, *O. kisutch*, *O. nerka, Cyprinus carpio*, *Lepomis macrochirus*, *Micropterus salmoides*, *Micropterus dolomieu*, *Pomoxis nigromaculatus*, *Cottus bairdii*, *Ictalurus punctatus*, *Catostomus columbianus*, *Ptychocheilus oregonensis*, *Prosopium williamsoni, Salmo trutta, Perca flavescens, Ameiurus sp., Richardsonius balteatus*) to directly test assay specificity. Fin clips were stored in 2-mL cryo-vials filled with 200 proof EtOH until DNA could be extracted using a Qiagen DNeasy Tissue & Blood Extraction Kit (Qiagen, Inc.), following the protocol included with the kit. All Chinook tissue samples produced positive detections using the Chinook assay, while none of the non-target tissue samples produced a positive detection with the exception of Coho salmon. However, 0.01X dilutions of Coho DNA extracted from fin clips, a concentration still higher than would likely be present in environmental samples, failed to amplify using the Chinook assay. Additionally, three 1-L water samples collected from Peterson Creek (Southeast, AK USA), a stream void of Chinook, but with a high-concentration of Coho (Johnson and Daigneault 2013) were tested using the Chinook assay and none of the samples tested positive for Chinook. A potential for cross-amplification of the Chinook assay in the

presence of extremely or unnaturally high concentrations of Coho DNA may be a concern if applied in systems where this is probable.

Sequencing Methods

Conventional PCR was performed on DNA extracted from Chinook fin clips (n=5) using a Tetrad PTC-225 Thermo Cycler (MJ Research, Inc.) and the same Chinook primer set used in qPCR to allow for sequencing to ensure that the PCR product contained the intended target sequence. Each reaction well consisted of 6.66 µl H2O, 10.5 µl Qiagen MasterMix (2X), 0.42 µl F-primer (10 µm concentration), 0.42 µl R-primer (10µm concentration), and 3 µl Chinook DNA extract. PCR cycling conditions were 15 minutes initial denaturation at 95C followed by 35 cycles of [30 seconds denaturation at 94C, 90 seconds annealing at 58-50C, and 60 seconds elongation at 72C] followed by 30 minutes final elongation at 60C. The PCR product was screened on 1% agarose gel using 120-121 volts along with a 1 kb ladder. The PCR product was then bi-directionally sequenced to ensure comprehensible sequence data. Sequencher 5.0 software (Gene Codes Corp.) was used to call individual bases.

The PCR product from a sub-set of field samples (n=15) were also sent to GeneWiz (GeneWiz Co.), where they were purified using ExoSAP-IT (Affymetrix, Inc.) and sequenced using Sanger sequencing to verify that the PCR product obtained from field samples was comprised of our intended target sequence.

Field-Sample DNA Extraction Procedure

We extracted DNA from filter samples using a Qiagen DNeasy Tissue and Blood extraction kit (Qiagen, Inc.) (Goldberg *et al.* 2011, Pilliod *et al.* 2013a, Pilliod *et al.*

2013b). The supplied protocol was followed except for the following modifications (Pilliod *et al.* 2013a): Before extraction, the filters were removed from the ethanol-filled vials and cut in half using sterile forceps. One half of the filter was used for extraction, the other half was placed back in the ethanol-filled vials and stored at -20C for archiving, or to be used as a back-up in case the first filter half became contaminated. The filter-half used for DNA extraction was placed into a sterile 2-mL flip-top vial with the lid left open for 24 hours to allow for evaporation of all ethanol. After 24 hours, 180 µl ATL buffer and 20 µl proteinase-K were added to lyse cells. Filter material was pushed down into the solution using a pipette tip and vortexed to ensure coverage. Vials were incubated at 55C for 24 hours. Using sterile forceps, filter material was then removed from flip-top vials and transferred to a QiaShredder spin column. Remaining solution was transferred as well, using a sterile filter-tip pipette. QiaShredder spin columns were centrifuged at 8000RPM for 3 minutes to separate the filter from the DNA-containing solution. The solution was then transferred to the spin column included in the DNeasy kit and Qiagen protocol was followed for remainder of DNA extraction except that only 100 µl of AE elution buffer was used in order to further concentrate DNA for final storage.

qPCR Procedure

Primers and probe were centrifuged and re-suspended in appropriate amounts of AE buffer to make 100 µM stock solutions. 20X primer/probe mix working stocks (4 µM concentration) were created using 8 µl each F-primer, R-primer, and MGB-probe, plus 176 µl AE buffer in a UV-sterilized 2-mL flip-top tube. Specificity of our molecular assay required an increased annealing temperature of 70C, at which we had difficulty incorporating our Taq-Man Exogenous Internal Positive Control Reagents (EXO-IPC)

(Life Technologies, Corp.), which are optimized for an annealing temperature of 60C. Therefore, samples were run initially at 70C, without inclusion of an internal positive control, and any samples that failed to amplify were then re-run at 60C with 0.6 µl 10X EXO-IPC and 0.3 µl 50X EXO-IPC (substituted for its corresponding volume of water) to check for inhibition. Eventually, we opted to utilize the internal control assay and template included with Qiagen Quantifast Pathogen PCR + IC Kit, an internal positive control that will amplify at the increased annealing temperature (70C), and this was then integrated into each reaction for the remainder of the samples. The three variations of qPCR reactions and cycling conditions used were as follows: Samples without an integrated internal positive control: 3.75 µl H20, 7.5 µl Quantitect MasterMix (2X), 0.75 µl primer/probe mix (20X), and 3 µl DNA extract for a 15 µl total reaction volume per well. Cycling conditions for qPCR were 15 minutes PCR initial heat activation at 95C, 60 seconds denaturing at 94C, 60 seconds annealing/extension at 70C repeated for 50 cycles. Samples that were re-run to check for inhibition: 3.6 µl H20, 7.5 µl Quantitect MasterMix (2X), 0.6 µl EXO-IPC (10X), 0.3 µl EXO-IPC (50X), and 3 µl DNA extract for a 15 µl total reaction volume per well. Cycling conditions for qPCR were 15 minutes PCR initial heat activation at 95C, 60 seconds denaturing at 94C, 60 seconds annealing/extension at 60C, repeated for 50 cycles. Samples that were run with an integrated internal positive control (preferred method): 2.25 µl H20, 7.5 µl Quantitect MasterMix (2X), 0.75 µl primer/probe mix (20X), 0.75 µl IC assay, 0.75 µl IC template, and 3 µl DNA extract for a 15 µl total reaction volume per well. Cycling conditions for qPCR were 15 minutes PCR initial heat activation at 95C, 60 seconds denaturing at 94C, 60 seconds annealing/extension at 70C, repeated for 50 cycles. Data were collected during the

annealing/extension step for all reactions. A standard curve was calculated using DNA extracted from Chinook tissue, quantified using a NanoDrop (Thermo Fisher Scientific Inc.), and then included in serial dilution (10^{-2} thru 10^{-6}) with each plate. All plates also contained one well with 3 µl DNAse/RNAse-Free H2O, rather than DNA, as a PCR non-template control (NTC). Samples were run on 96-well clear optical plates on ABI 7300 and ABI 7500FAST Real-time PCR Systems (Life Technologies, Corp.). All field samples were run in triplicate (pipetted into 3 separate wells, with values averaged for each sample) to ensure detection of degraded or low-quantity DNA (Waits & Paetkau 2005). Any samples that showed signs of inhibition were diluted 0.1X, to reduce inhibitors, and re-run (Pilliod *et al.* 2013a). Analysis of qPCR data was conducted using AB Sequence Detection Software (Version 1.4.0.25, Applied Biosystems, Inc.).

Data Analysis

To determine if our eDNA detection methods were effective in determining the distribution of Chinook, we compared eDNA detection results at sites selected *a priori* as Chinook likely present (those that have had Chinook detection using traditional survey methods) to sites selected as Chinook likely absent (where Chinook have not been detected using traditional survey methods). A Pearson's Chi-squared test (with Yates' continuity correction) was used to test whether the effectiveness of our eDNA detection were due to chance or likely a result of the sensitivity of the method.

Detection probability (ρ) was calculated as the sum ($\sum$) of individual site detection probabilities (ρ_i) over the number of sites (i), ± standard error (SE), or: $\rho = (\sum \rho_i / i) \pm SE$. Individual site detection probabilities were calculated as the number of 1-L replicates that tested positive for Chinook eDNA (0-3) at a site divided by the number of

replicates collected at that site (3). This was calculated for the high flow (June) and low flow (August) sampling events separately, as well as a combined.

We expected eDNA concentrations to decrease as distance upstream increased, due to fewer Chinook inhabiting the higher headwater tributaries, and also due to a downstream accumulation of genetic material. We tested this using 3 sets of sites, one in the Upper-Methow watershed (*Sites 15, 18, 21, 23*), one in the Twisp watershed (*Sites 6, 8, 10*), and another in the Chewuch watershed (*Sites 24, 27, 29, 31, 32*) (Figure 2.2). Distance upstream was measured from the lowest site in each set, using ArcGIS v10.1 software (ESRI Inc.), with lowest sites receiving distance 0 km. A regression analysis was used to determine if a negative linear relationship exists between eDNA concentration and the distance upstream of the sample site (i.e., if the β of the best-fit-line $\neq 0$).

Chinook eDNA concentrations collected during high flow (June) and low flow (August) were compared using the mean from both sampling periods (Methow and Okanogan combined). A non-parametric, Wilcoxon signed rank test (with continuity correction) was used to determine a shift in means between the June and August samples.

To examine the rate of false-negatives (and the number of necessary 1-L replicates that should be collected at each site for future eDNA studies), the number of sites in which 0, 1, 2, and 3 replicates tested positive for Chinook eDNA were summed. Each value was then divided by the total number of sites (n=96) to determine the percentage of sites for each category (0, 1, 2, or 3). Our rate of false-negatives was then the number of replicates in which no Chinook eDNA was detected (n=12) divided by the

total number of replicates collected at sites where Chinook were confirmed present (i.e., all sites where Chinook were detected in at least one replicate) (n=162).

To examine the relationship of eDNA concentration at sample sites above the approximate lethal water temperature for spring-Chinook, 18C (Marine 1992, Berman 1990), a model was fit to eDNA concentration and water temperature for samples with temperatures between 18C – 25.5C (n=32).

Two sites were omitted from statistical analysis: *Site 40 - Bonaparte Creek* (Okanogan Sub-basin) due to PCR inhibition and *Site 48 – Shingle Creek* (Okanogan Sub-basin), which was likely compromised (see Discussion). Water samples were also collected from a juvenile spring-Chinook rearing tank at Winthrop National Fish Hatchery (Winthrop, WA USA) on 26 June 2012. These samples served only as stream positive-controls, and were also omitted from analysis.

Statistical analyses were performed using R-Statistical Software (Version 2.15.3, 2013-03-01, © 2013 The R Foundation for Statistical Computing), unless otherwise stated.

Results

Using eDNA detection methods on sites selected *a priori* as Chinook likely present and Chinook likely absent (within range, and outside of range, respectively), we detected Chinook in all 27 sites where they were expected to be present (Table 2.1). We detected Chinook in an additional 5 sites where they were not expected, or not known to inhabit (*Boulder Cr., Little Boulder Cr., Little Bridge Cr., Inkaneep Cr., Vaseux Cr.*). These additional sites were above no known barriers to anadromy. We did not detect Chinook

DNA in any of our field negative-controls (distilled water) (n=97), laboratory DNA extraction negative controls (n=23), or qPCR negative controls (n=20). Based on this, eDNA detection methods appear to be a sensitive detection method for determining the distribution of Chinook (X^2 = 25.2243, df=1, p-value <0.0001).

In both the Methow and the Okanogan Sub-basins, we found an increase in the percentage of sites that tested positive for presence of Chinook DNA between high flow (June) and low flow (August) sampling events (Table 2.2). Positive detections in the Methow Sub-basin increased by 50% from high flow to low flow sampling. Positive detections in the Okanogan Sub-basin increased by 33.3% from high flow to low flow.

Among sites that tested positive for Chinook eDNA during *both* the high flow and low flow sampling events, there was a higher mean concentration of Chinook eDNA during the low flow sampling event (V = 96, p-value <0.0001) (Figure 2.3).

Among sites within the known distribution of Chinook (i.e., given presence), our overall detection probability (ρ) was 0.83 (high and low flow combined), while our high flow detection probability was 0.69, and low flow detection probability was 0.98 (Table 2.3).

The highest concentrations of Chinook eDNA were collected at sites with a water temperature ranging from approximately 12C – 18C (Figure 2.4).

Our hypothesis that Chinook eDNA concentrations would decrease as distance upstream increased was supported by a set of sites in the Methow watershed ($F_{(1,10)}$=19.54, p-value<0.001, Adjusted R-squared: 0.6277) (Figure 2.5). This hypothesis was not supported in the Twisp watershed, where the opposite relationship existed

(Figure 2.6). Further, Chinook eDNA concentrations from sites in the Chewuch were high in both upstream and downstream sites relative to the middle reaches (Figure 2.7). There was an exponential decrease in Chinook eDNA concentrations at sites with water temperatures above 18C (Adjusted R-squared: 0.8574) (Figure 2.8).

With triplicate sampling (3, 1-L water samples collected per site, per sampling event), there were 4 possible site detection outcomes (Table 2.4). Among the triplicates from sites that produced at least one positive detection for Chinook eDNA during a sampling event (i.e., with confirmed presence, n=162), 12 or 7.07% replicates failed to detect Chinook eDNA (false-negatives).

Discussion

Our overall detection probability (0.83) and the detection of Chinook eDNA at all sites within their known range as well as 5 additional sites outside of their known range (which are all accessible to Chinook, in that they are above no known barriers to anadromy) suggests that eDNA detection may be an effective method of determining the distribution of Chinook throughout large watersheds. These additional detections likely indicate that the distribution of Chinook in these Sub-basins is slightly larger than previously described.

The high detection probability (especially during low flow – 0.98) suggests a high sensitivity of the method to species presence, as has been determined by other studies (Ficetola *et al.* 2008, Jerde *et al.* 2011, Goldberg *et al.* 2011, Takahara *et al.* 2012, and others). Reduced detection rates during the high-flow (June) sampling event (0.69) suggests that either higher flows dilute available eDNA (reducing probability of collecting DNA in our filters) or that Chinook were not present in certain tributaries

during June. We are unable to confirm a closed-system between these two sample dates and it is possible additional Chinook may be migrating into the system. Therefore, differences in our detection rates between June and August may reflect true differences in occurrence, rather than solely an increase in detection probability.

Sites that tested positive for Chinook DNA during the June sampling event (n=22), especially those in the smaller tributaries (Figures 2.9 and 2.10), rather than the main-stem Methow and Okanogan Rivers (such as *Lake Cr., Lost Cr., Gold Cr., Chewuch R., Twisp R., Gold R., and Upper-Methow R.* in the Methow Sub-basin and *Omak Cr*. and *Salmon Cr*. in the Okanogan Sub-basin), likely indicate the presence of spring-Chinook. These are likely either adults arriving on their early upstream migration or sub-yearling juveniles that had yet to migrate to the ocean. Although the Okanogan Sub-basin spring-Chinook population is currently listed as extirpated (ESA), there is evidence of tagged spring-Chinook straying from nearby Sub-basins into the Okanagan (J. Arterburn & B. Miller, CCT F&W biologists, personal communication). This could account for the early detections. However, fall-Chinook juveniles that fail to migrate to the ocean, potentially up to ~40% of males in a hatchery population (Larsen *et al.* 2004, Larsen *et al.* 2013), could potentially also contribute to detectable Chinook eDNA during the June sampling event in both basins.

The molecular assay used in this study was designed to detect the presence of Chinook salmon at the species-level. To better understand distributions of spring- vs. fall-Chinook within a Sub-basin, without relying on spatial and temporal assumptions, an assay capable of accurately differentiating between the two life-history types, perhaps targeting SNP's (single-nucleotide polymorphisms) in additional subunits of the Chinook

genome, would be a necessary and valuable tool. Use of next-generation sequencing in eDNA studies, which essentially sequences all available DNA in a sample, has shown potential to identify a wide range of species (Thomsen *et al.* 2012b), and may also be effective in differentiating run-types or populations within a basin.

Our northernmost site, *Site 48 – Shingle Cr.*, located outside of the current known distribution of Chinook (although within its historical range), and above known barriers to anadromy, tested positive for the presence of Chinook DNA. Shingle Creek is a well-studied stream, which flows through farm and ranchland and eventually the Penticton Indian Band community lands (Walsh & Long 2005 - manuscript status) before joining the Okanogan River between Okanogan and Skaha Lakes. Sequence data of amplified DNA confirmed presence of Chinook DNA. Presence of Chinook DNA at this site is unlikely to have occurred through natural distribution, due to barriers and that no Chinook have been detected in annual surveys in recent times (Benson & Squakin 2008 – manuscript status). This suggests potential site contamination, not through sampling equipment (as no Chinook DNA was detected in field negatives), but possibly through artificial introduction of Chinook genetic material into the stream (carcass disposal or unreported introduction/release of live fish). While not greatly affecting the results of this study (as this site was omitted from analysis), this does draw attention to the need to accurately interpret results of this highly-sensitive, sight-unseen detection method. Especially when targeting a widely-distributed and highly sought after sport-fish in a study area with a high human population, the potential for transporting genetic material is very real and should be considered whenever unlikely results or conclusions are encountered.

Samples collected at spring-Chinook rearing tanks at the US Fish & Wildlife Service (USFWS) Winthrop National Fish Hatchery (WNFH) produced the highest eDNA concentrations throughout the study (Appendix B). This tank held high densities of juvenile spring-Chinook at the time of sampling. These tanks were designed with a flow through system, allowing water to flow in from the Upper-Methow River, through the tanks, and then through an outflow just below the hatchery, feeding back into the Methow River. Reduced DNA concentrations at sites successively further downstream of this hatchery 'input' of Chinook genetic material suggest that there is a rather rapid reduction in DNA concentration downstream of this source, rather than a simple downstream accumulation (Figure 2.9). This rapid breakdown is confirmed by other studies (Dejean *et al.* 2011, Thomsen *et al.* 2012a, Thomsen *et al.* 2012b, Pilliod *et al.* 2013a). Our 3 sets of sites designed to test the hypothesis of downstream accumulation produced mixed results (Figure 2.5, 2.6, and 2.7). The Methow watershed set showed an increase in eDNA concentration lower in the system, which could be due either to our hypothesized downstream accumulation of eDNA or possibly the presence of larger numbers of fall-Chinook lower in the system, with fewer spring-Chinook towards the headwaters. Fall-Chinook are known to use the lower end of the Upper-Methow River, near the confluence with the Chewuch River (UCSRB 2007). The opposite relationship existed in the Twisp watershed, with eDNA concentration increasing with distance upstream. This suggests that greater numbers of Chinook were present higher up, and that eDNA was degrading beyond recognition or possibly adsorping to material not being carried downstream in suspension. The curvi-linear relationship between eDNA concentration and distance upstream in the Chewuch watershed suggests that more

Chinook may have been present both in the uppermost extent as well as in the lowest extent of the watershed, with fewer fish in between. Downstream accumulation of eDNA did not appear to be influencing this relationship. As in many stream-based studies, without confirmation of a closed-system or independence of sites, we are limited in our ability to confidently resolve the cause of these results (Fausch *et al.* 1988, Dunham & Vinyard 1997). For example, one confounding factor is the input of tributaries between these sites, which may be either adding to the Chinook eDNA concentration, or simply diluting. Whether due to dilution, mechanical breakdown, organic digestion or possible adsorption of DNA molecules as they flow downstream through the environment, this indicates a difficulty associated with attempting to use quantitative eDNA concentrations to infer upstream population densities. Because of this rapid loss of genetic material in aquatic environments and our lack of evidence to support a general model, we were only able to infer relative densities of Chinook between sites, rather than attempt to calculate any actual density based on eDNA concentrations.

Water temperature has been shown to affect both the degradation rate of eDNA (Pilliod *et al.* 2013b), as well as the likelihood of Chinook presence, in terms of habitat suitability (Brett 1952, Coutant 1977). Our results show highest eDNA concentrations at sites within the ideal water temperature range of the species (Figure 2.4), and an exponential decrease in Chinook eDNA concentrations from 18C to 25.5C (our highest water temperature), which is similar to results from studies using traditional detection methods that found a linear decrease in rearing densities of several salmonids above 17C (Frissell 1992). While our results are somewhat confounded by the increased rate of DNA

degradation that occurs at higher temperatures, this may suggest a potential use of eDNA detection to help determine or confirm habitat suitability across large habitats.

To reduce false-negatives, studies using eDNA detection have tended to collect water in triplicate at a sample site (Ficetola *et al.* 2008, Goldberg *et al.* 2011, Pilliod *et al.* 2013a, Pilliod *et al.* 2013b). We found that with confirmed presence of Chinook eDNA at a site, 7.07% of our replicate 1-L water samples produced false-negatives (Table 2.4). This suggests that, depending on the application, fewer samples could be collected at a site to reduce the cost of eDNA sampling by up to two-thirds (1 sample vs. 3), while still maintaining a reasonably low rate of false-negatives.

Our baseline survey of Chinook presence in the Okanogan Sub-basin shows distribution primarily along the main-stem Okanogan, typical of fall-Chinook distributions, with occasional occurrence in some of the larger tributaries as mentioned (Figures 2.11 and 2.12). This baseline data will serve as an initial monitoring survey, prior to the re-introduction of spring-Chinook to the Okanogan by the Colville Confederated Tribes. With future monitoring, and by comparing back to these survey data, they should be able to track changes in Chinook distribution following the re-introduction, helping them to assess its progress.

This study will hopefully provide insight to help develop monitoring programs using eDNA to determine the distribution of salmonids in large watersheds. For the purpose of population monitoring, this method is not necessarily intended to replace traditional survey methods such as electrofishing or snorkel count surveys (especially where count data or actual-, rather than relative-density is required) but could be a

valuable, complimentary tool to rapidly determine distributions and assess and prioritize stream reaches to better assign limited resources.

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Table 2.1. eDNA detection vs. distribution determined using traditional survey methods

	eDNA methods		
Known distribution[1]	Detected	Not-detected	Number of sites
Chinook likely-present	27	0	27
Chinook likely-absent	5	14	19

Note[1]: 'Known distribution' adapted from Upper-Columbia River Salmon Recovery Board Spring-Chinook and Steelhead Recovery Plan 2007 (UCSRB 2007) and personal communication with regional fisheries biologists

Table 2.2. eDNA sample sites that tested positive for Chinook DNA during high and low flow

	(+) eDNA sites		
	High flow	Low flow	Increase
Methow	16 of 32	24 of 32	50%
Okanogan	6 of 14	8 of 14	33.3%
Total number of sites	22 of 46	32 of 46	45.5%

Table 2.3. eDNA detection probability ± SE

	Detection probability	±SE
High flow (June)	0.69	0.08
Low flow (August)	0.98	0.02
Combined	0.83	0.04

Table 2.4. Number of 1-L water samples that tested positive for presence of Chinook DNA, based on triplicate sampling at each sample site.

Possible detection outcomes at a site[1]	% of sites	# replicates (-)	# replicates total
0 0 0	41%	-	-
1 0 0	3%	6	9
1 1 0	7%	6	18
1 1 1	49%	0	135
Total	100%	12	162

Note[1]: 0 = No Chinook eDNA detected, 1 = Chinook eDNA detected

Figure 2.1. Study area -Methow and Okanogan Sub-basins in North-central Washington State, USA and Southern British Columbia, Canada.

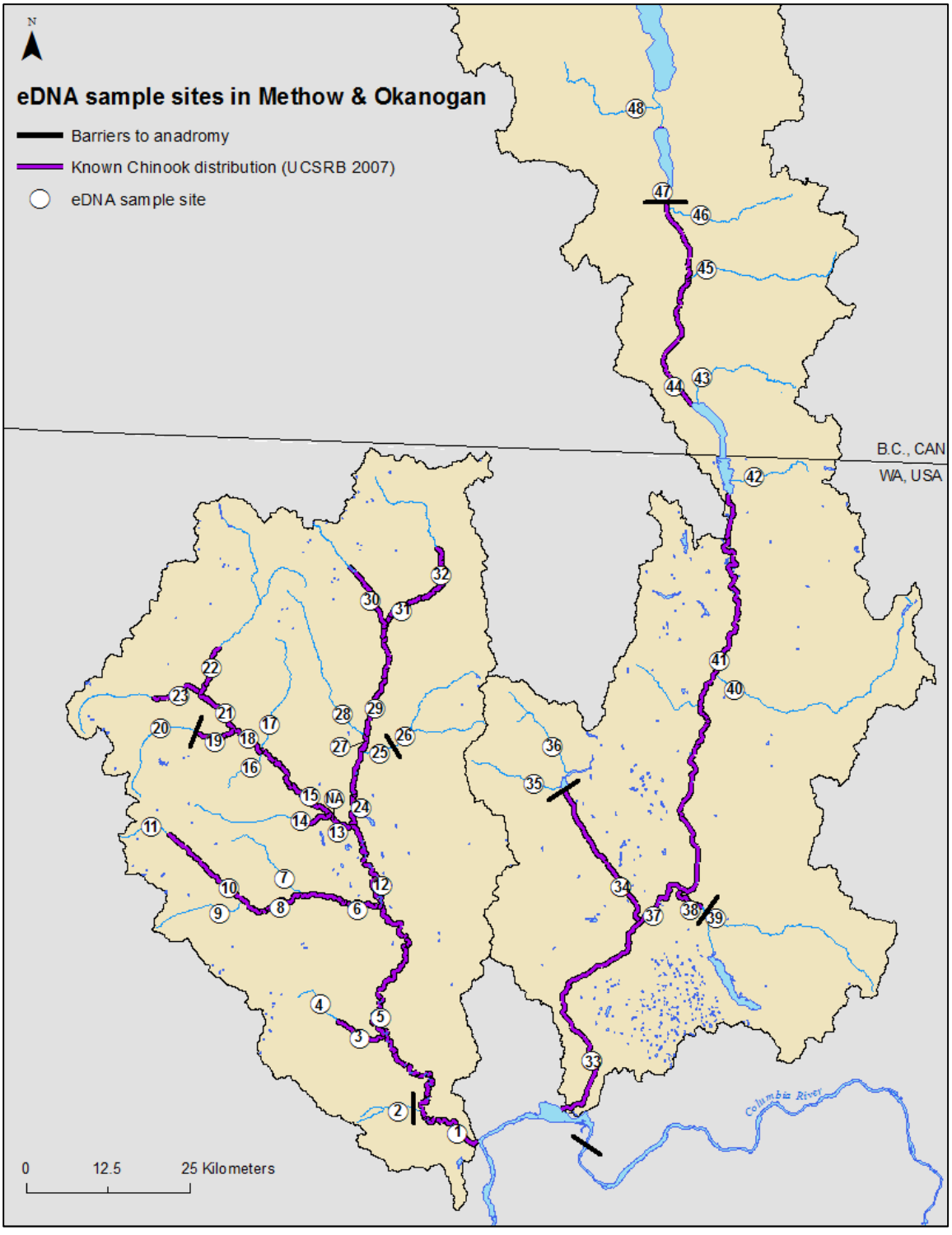

Figure 2.2 eDNA sample sites (numbered) throughout Methow and Okanogan Sub-basins

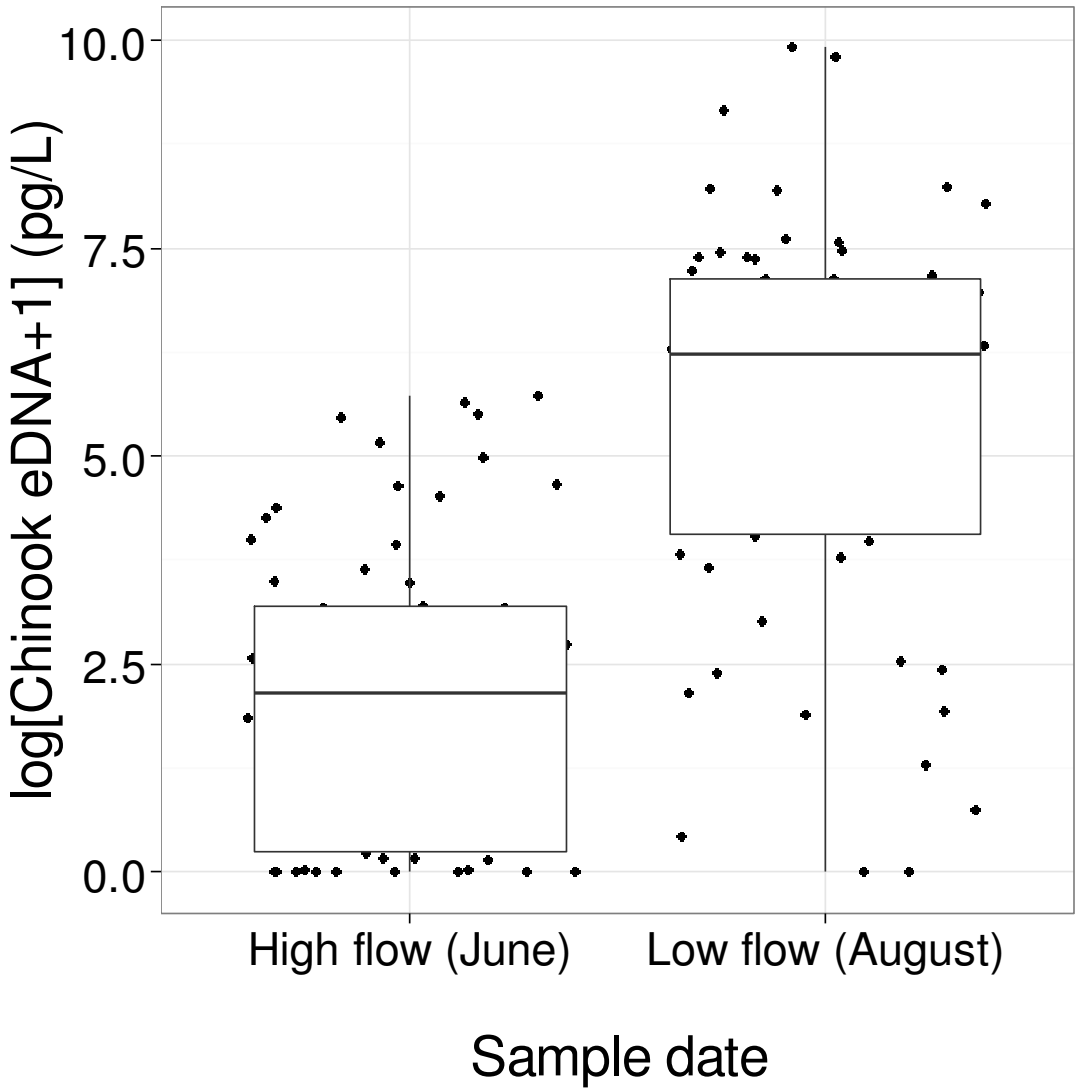

Figure 2.3 Mean Chinook eDNA concentration (pg/L) for June and August sampling events (V = 96, p-value <0.0001).

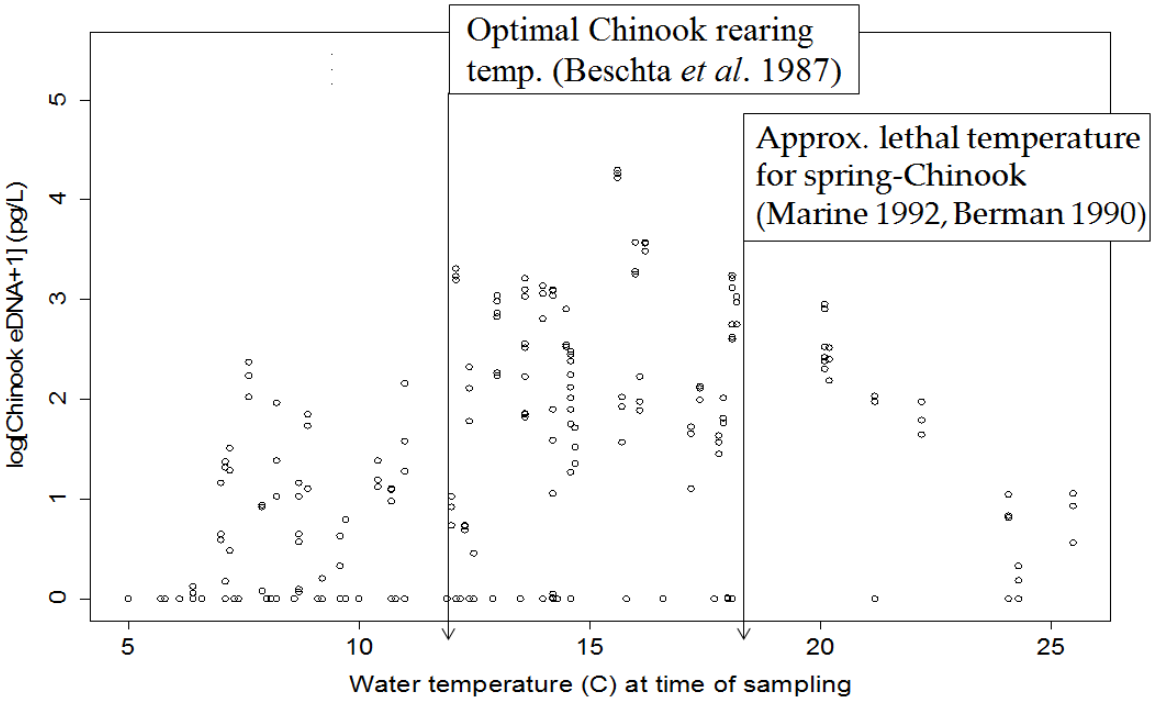

Figure 2.4. eDNA concentration by water temperature at time of sampling.

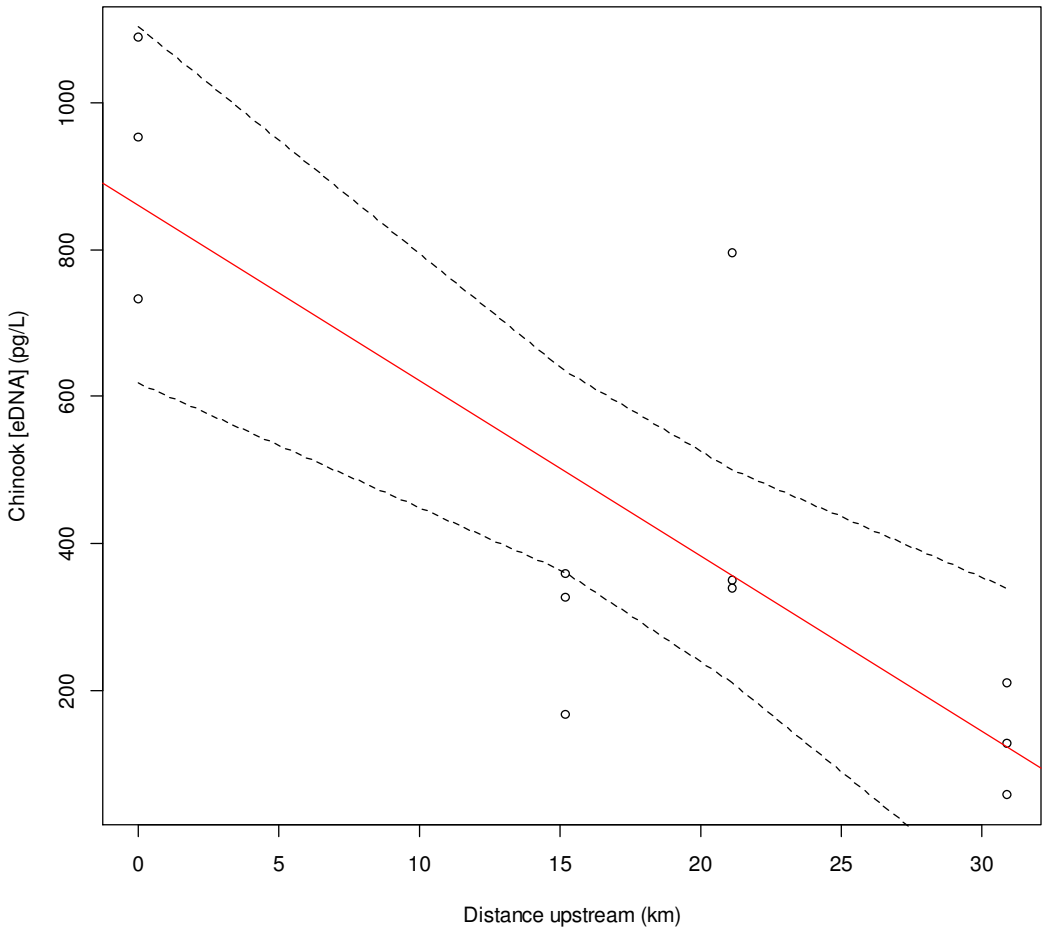

Figure 2.5. Downstream accumulation of Chinook eDNA in the Upper-Methow watershed. Dashes lines represent 95% confidence intervals. F-statistic: 19.54 on 1 and 10 DF, p-value: 0.001292 Adjusted R-squared: 0.6277

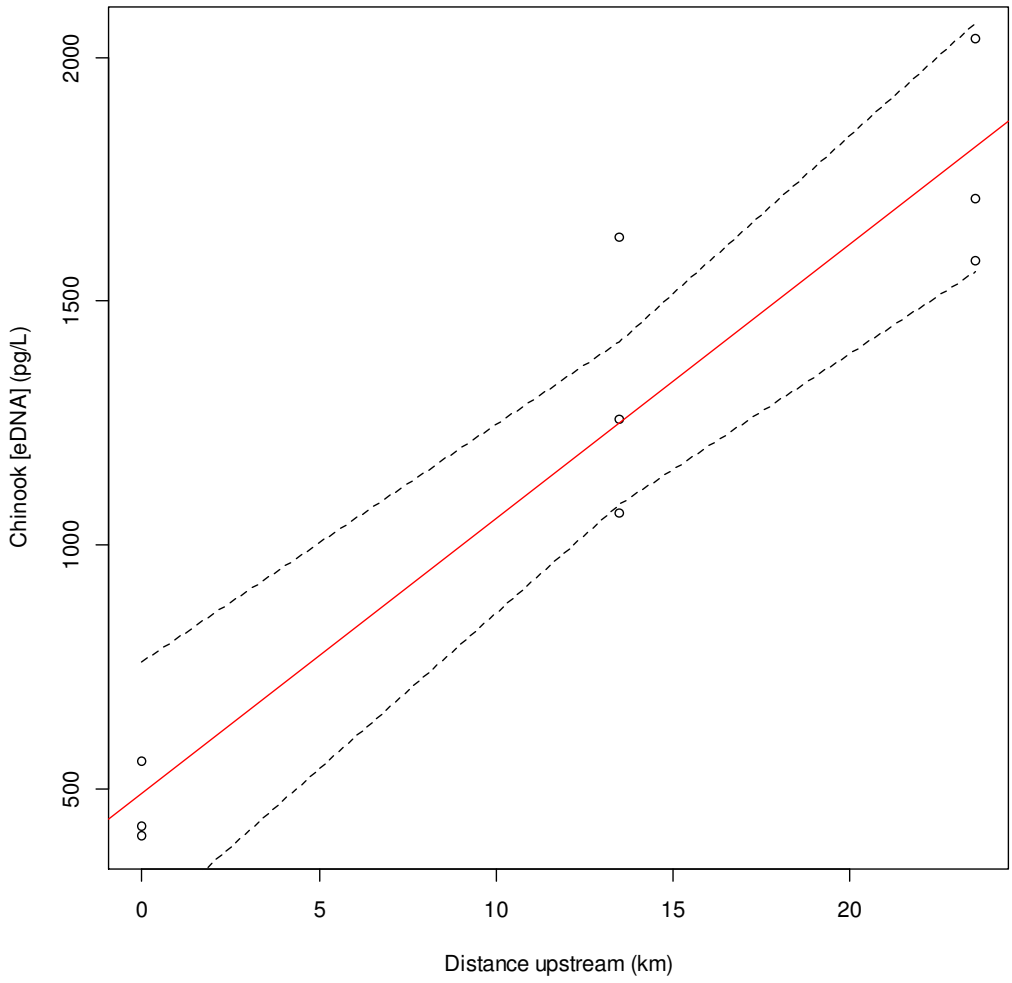

Figure 2.6. Downstream accumulation of Chinook eDNA in the Twisp watershed. Dashed lines represent 95% confidence intervals.

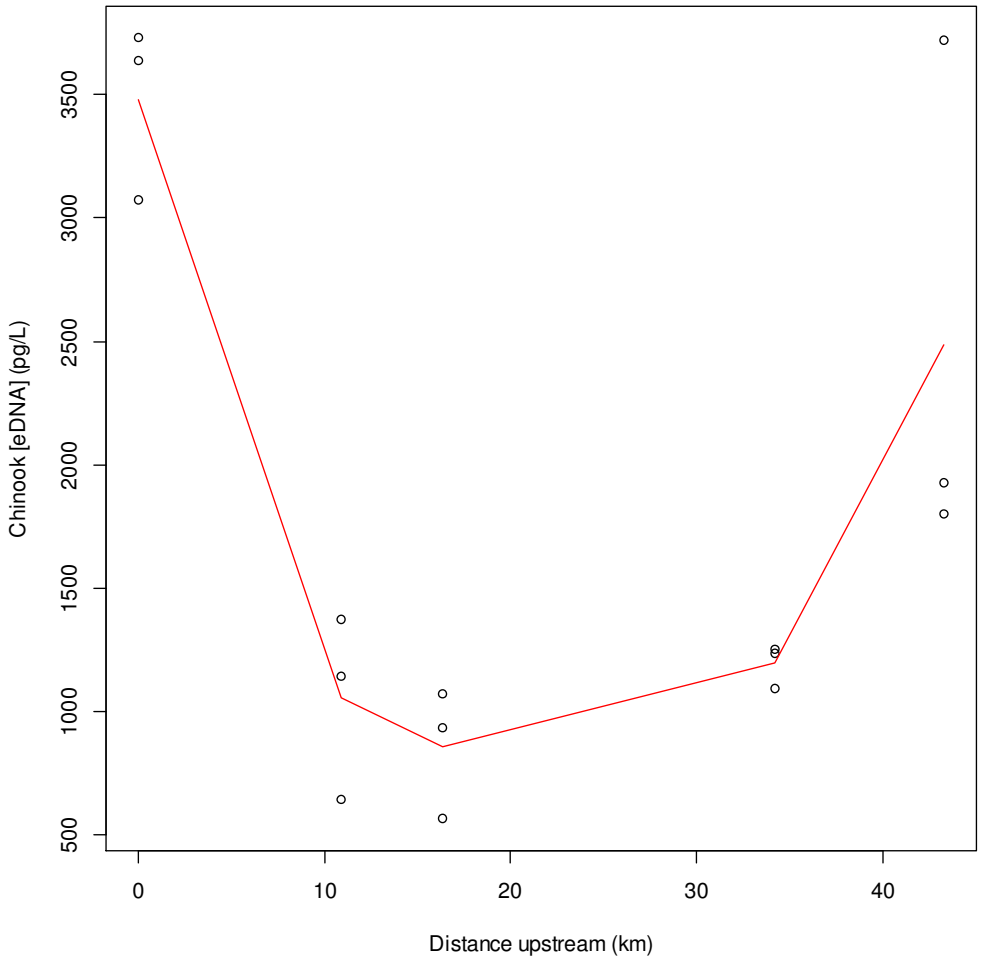

Figure 2.7. Downstream accumulation of Chinook eDNA in the Chewuch watershed

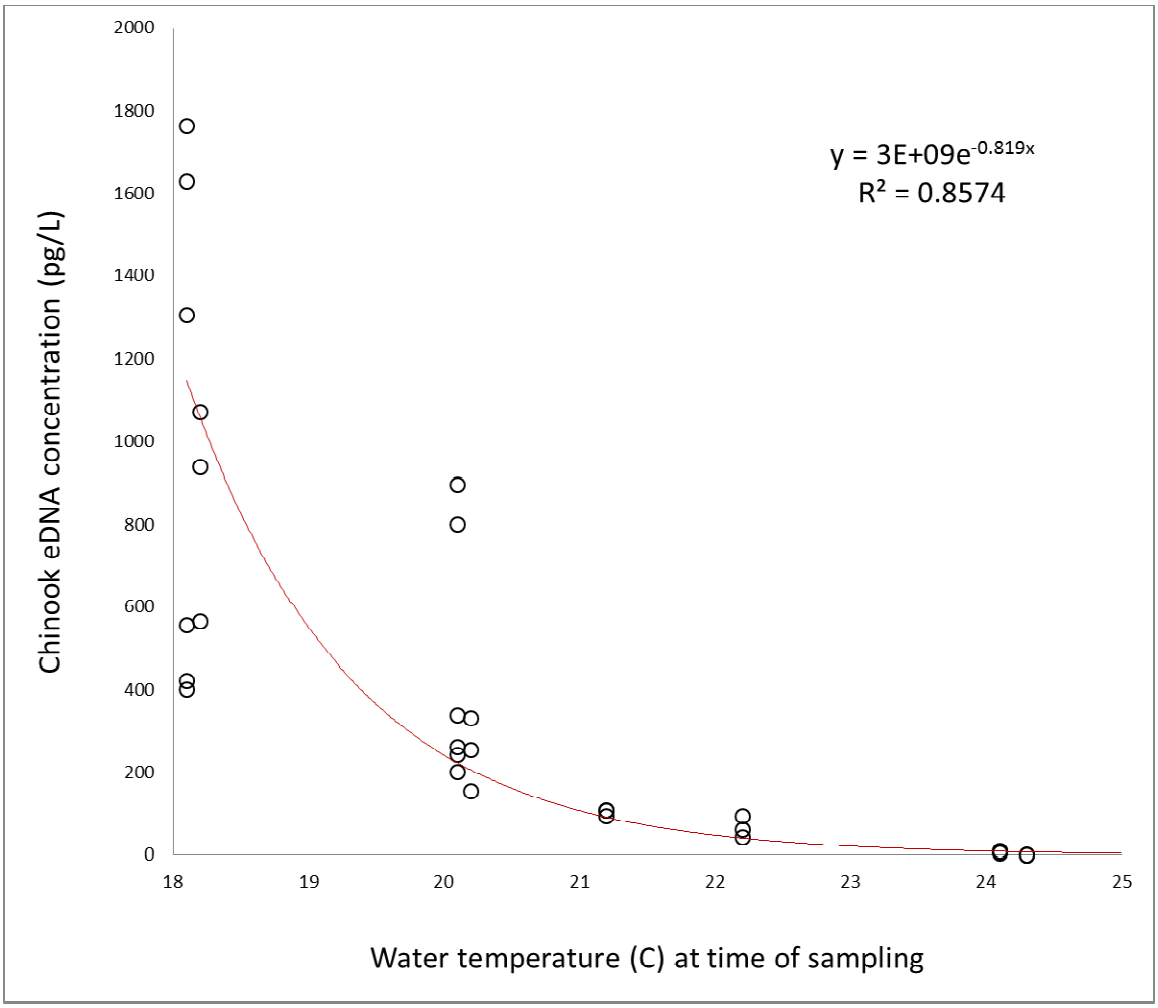

Figure 2.8. Exponential decrease in Chinook eDNA concentration at sites with water temperatures above 18 C.

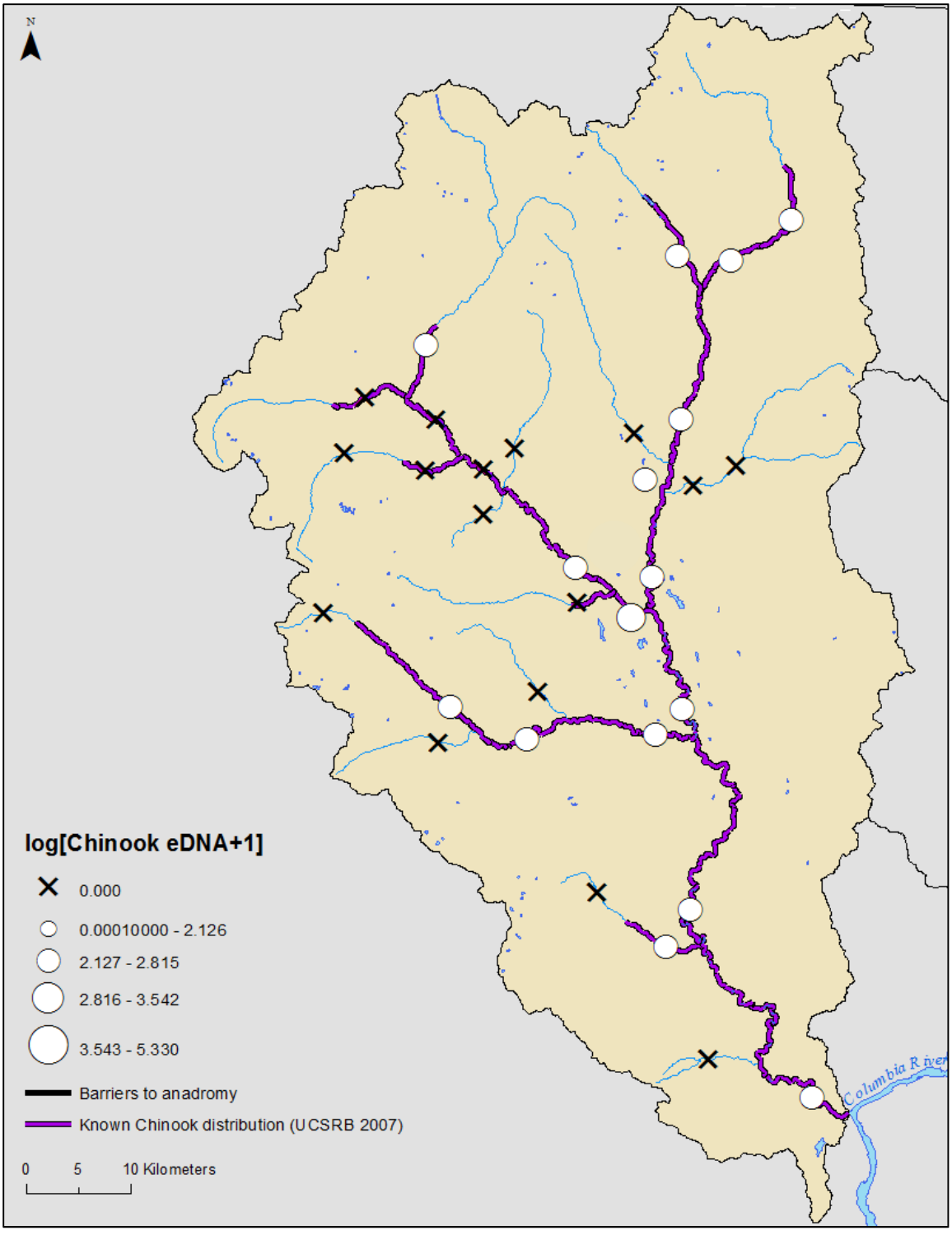

Figure 2.9. High flow (June) Chinook eDNA concentrations in the Methow Sub-basin

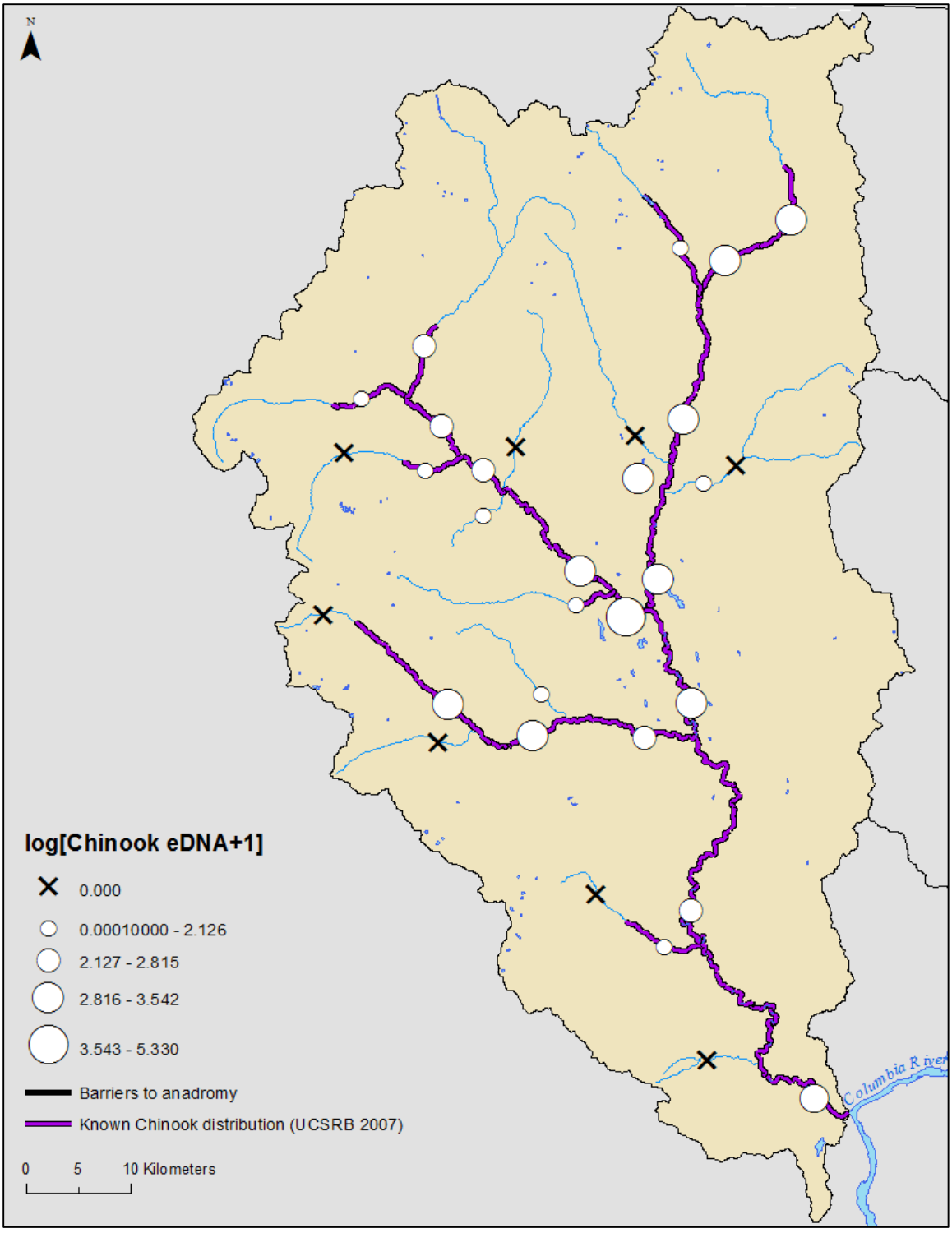

Figure 2.10. Low flow (August) Chinook eDNA concentrations in the Methow Sub-basin

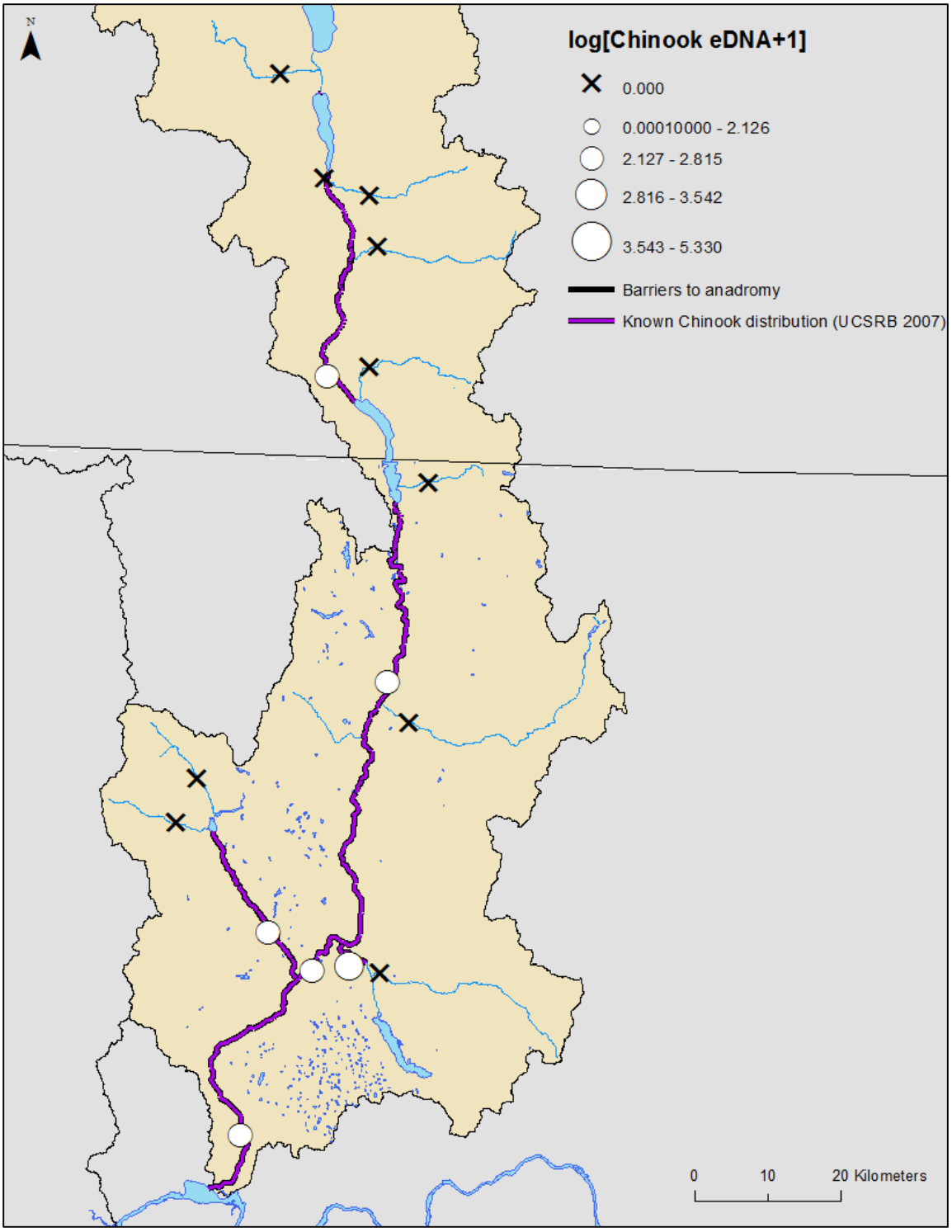

Figure 2.11. High flow (June) Chinook eDNA concentrations in the Okanogan Sub-basin

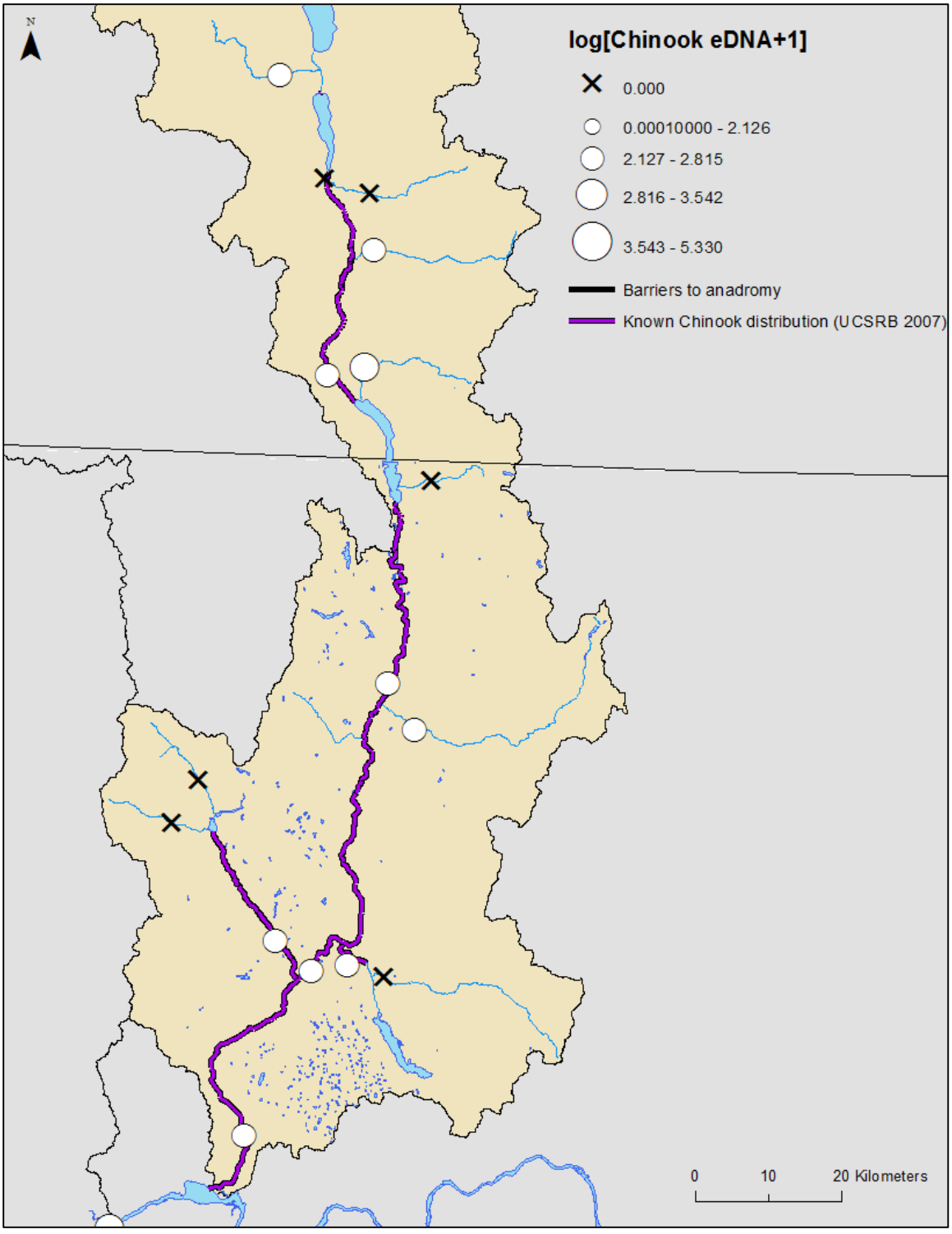

Figure 2.12. Low flow (August) Chinook eDNA concentrations in the Okanogan Sub-basin

APPENDIX A

Chinook eDNA Sample Sites List for Methow and Okanogan Sub-basins with Stream Names, Sub-Basin and Coordinates (UTM, DATUM: NAD83)

APPENDIX A

Table A.1 Chinook eDNA sample sites list for Methow and Okanogan Sub-basins with stream names, Sub-basin and coordinates (UTM, DATUM: NAD83)

GIS

SITE	STREAM	SUBBASIN	UTM_ZONE	UTM_NORTH	UTM_EAST
1	Methow River, Lower	Methow	11	282913	5325451
2	Squaw Creek	Methow	10	721837	5330610
3	Gold Creek	Methow	10	715757	5341324
4	Gold Creek	Methow	10	706242	5344326
5	Methow River, Lower	Methow	10	715099	5341737
6	Twisp River	Methow	10	711148	5361240
7	Little Bridge Creek	Methow	10	700954	5362044
8	Twisp River	Methow	10	700681	5361938
9	War Creek	Methow	10	692176	5360060

10	Twisp River	Methow	10	692180	5360506
11	Twisp River	Methow	10	679032	5370432
12	Methow River, Lower	Methow	10	711972	5363867
13	Methow River, Upper	Methow	10	707632	5372837
14	Wolf Creek	Methow	10	704532	5374484
15	Methow River, Upper	Methow	10	704102	5374960
16	Little Boulder Creek	Methow	10	693248	5382942
17	Goat Creek	Methow	10	693249	5384231
18	Methow River, Upper	Methow	10	691186	5385121
19	Early Winters Creek	Methow	10	688576	5386155
20	Early Winters Creek	Methow	10	677768	5385638
21	Methow River, Upper	Methow	10	687457	5388680

22	Lost River	Methow	10	683679	5392124
23	Methow River, Upper	Methow	10	680741	5392409
24	Chewuch River	Methow	10	707919	5373222
25	Boulder Creek	Methow	10	709057	5384485
26	Boulder Creek	Methow	10	710619	5385478
27	Chewuch River	Methow	10	708336	5384490
28	Eight Mile Creek	Methow	10	708672	5387426
29	Chewuch River	Methow	10	709270	5388120
30	Lake Creek	Methow	10	710510	5404321
31	Chewuch River	Methow	10	710657	5404356
32	Chewuch River	Methow	10	718647	5411365
33	Okanogan River	Okanogan	11	301312	5338172

34	Salmon Creek	Okanogan	11	305705	5364858
35	West Fork Salmon Creek	Okanogan	11	295574	5379968
36	North Fork Salmon Creek	Okanogan	11	296183	5385254
37	Okanogan River	Okanogan	11	308781	5359504
38	Omak Creek	Okanogan	11	314550	5363449
39	Omak Creek	Okanogan	11	320138	5360062
40	Bonaparte Creek	Okanogan	11	320530	5396899
41	Okanogan River	Okanogan	11	319389	5396177
42	Nine Mile Creek	Okanogan	11	323026	5427017
43	Inkaneep Creek	Okanogan	11	317309	5439132
44	Okanogan River	Okanogan	11	314955	5440448
45	Vaseaux Creek	Okanogan	11	316247	5457788

46	Shuttleworth Creek	Okanogan	11	313215	5468581
47	Okanogan River	Okanogan	11	312498	5468339
48	Shingle Creek	Okanogan	11	311247	5484038
NA	Winthrop Ntl Fish Hatchery	Methow	10	707698	5372768

APPENDIX B

Chinook eDNA Concentrations for Sample Sites in Methow and Okanogan Sub-Basins in June and August

APPENDIX B

Table B.2 Chinook eDNA concentrations for sample sites in Methow and Okanogan Sub-basins in June and August

GIS SITE	STREAM	SITE	[eDNA]_JUNE	SD	[eDNA]_AUGUST	SD
1	Methow River, Lower	MET1	66.11998668	67.47425957	652.202	342.577532
2	Squaw Creek	SQUAW1	0	0	0	0
3	Gold Creek	GOLD1	5.074639778	4.235193164	36.10181778	21.49290057
4	Gold Creek	GOLD2	0	0	0	0
5	Methow River, Lower	MET2	44.69828444	29.60890099	261.0346667	70.78318185
6	Twisp River	TWISP1	17.19050711	14.62709274	460.2613333	83.45513501
7	Little Bridge Creek	LTLBRIDGE1	0	0	4.225428889	0.291347743
8	Twisp River	TWISP2	6.57806	5.958358211	1317.699111	287.6160742
9	War Creek	WAR1	0	0	0	0

10	Twisp River	TWISP3	14.26483934	12.02107927	1776.241556	235.0318044
11	Twisp River	TWISP4	0	0	0	0
12	Methow River, Lower	MET3	41.32832666	43.74111668	1566.649111	234.8850433
13	Methow River, Upper	MET4	170.6793778	64.60976775	18267.45556	1821.633905
14	Wolf Creek	WOLF1	0	0	111.9651111	48.70271179
15	Methow River, Upper	MET5	1.72478	2.987406592	925.1431112	179.7100173
16	Little Boulder Creek	LITBOULD1	0	0	3.504615557	5.949733908
17	Goat Creek	GOAT1	0	0	0	0
18	Methow River, Upper	MET6	0	0	284.4971556	102.0395877
19	Early Winters Creek	EARLY1	0	0	69.03655556	2.985346748
20	Early Winters Creek	EARLY2	0	0	0	0
21	Methow River, Upper	MET7	0	0	494.7715556	260.8237571

22	Lost River	LOST1	0.167175333	0.175610364	341.8356222	283.1614076
23	Methow River, Upper	MET8	0	0	132.7411556	76.0717261
24	Chewuch River	CHEW1	1.038703556	1.440380602	3479.637778	356.0261876
25	Boulder Creek	BOULDER1	0	0	75.1106889	35.6143441
26	Boulder Creek	BOULDER2	0	0	0	0
27	Chewuch River	CHEW2	16.7107	5.924924979	1055.733556	372.3097359
28	Eight Mile Creek	EIGHT1	0	0	0	0
29	Chewuch River	CHEW3	10.53803111	1.815471377	857.7488888	261.8640331
30	Lake Creek	LAKE1	0.410354667	0.355377566	120.9594667	61.44641382
31	Chewuch River	CHEW4	8.842898668	5.102332169	1196.210444	87.87625638
32	Chewuch River	CHEW5	1.447305111	1.631483863	2484.136222	1074.085236
33	Okanogan River	OK1	34.79852667	14.46091592	6.830168666	3.877871863

34	Salmon Creek	SAL1	7.098224444	2.528303892	38.78902222	39.13367006
35	West Fork Salmon Creek	WFSAL1	0	0	0	0
36	North Fork Salmon Creek	NFSAL1	0	0	0	0
37	Okanogan River	OK2	0.615382222	1.065873275	7.14032	2.483785272
38	Omak Creek	OMAK1	275.3406667	30.52446661	102.3889333	7.484353919
39	Omak Creek	OMAK2	0	0	0	0
40	Bonaparte Creek	BONA1	NA	NA	119.5728444	19.31522452
41	Okanogan River	OK3	66.24564445	43.99312242	65.47666667	25.54594582
42	Nine Mile Creek	NINE1	0	0	0	0
43	Inkaneep Creek	INKA1	0	0	246.035	88.8228906
44	Okanogan River	OK4	0.011533333	0.011184712	0.548464667	0.561621598
45	Vaseaux Creek	VAS1	0	0	35.05740444	7.338762936

46	Shuttleworth Creek	SHUT1	0	0	0	0
47	Okanogan River	OK5	0	0	0	0
48	Shingle Creek	SHING1	0	0	74.18966666	23.93013366
NA	Winthrop Ntl Fish Hatchery	HATCHERY1	213600.1778	72935.48757	NA	NA