

A RAPID ASSESSMENT METHOD TO ESTIMATE THE DISTRIBUTION OF JUVENILE
CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) IN
AN INTERIOR ALASKA RIVER BASIN

By

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Abstract

Identification and protection of water bodies used by anadromous species in Alaska are critical in light of increasing threats to fish populations, yet challenging given budgetary and logistical limitations. Non-invasive, rapid assessment sampling techniques may reduce costs and effort while increasing species detection efficiencies. I used an intrinsic potential (IP) habitat model to identify high quality Chinook Salmon *Oncorhynchus tshawytscha* rearing habitats and select sites to sample throughout the Chena River basin for juvenile occupancy using environmental DNA (eDNA) and distribution within tributaries using snorkel surveys. Water samples were collected from 75 tributary sites in 2014 and 2015. The presence of Chinook Salmon DNA in water samples was assessed using a quantitative polymerase chain reaction (qPCR) assay targeting that species. Snorkel surveys were conducted and physical habitat was measured for a subset of tributaries examined with the eDNA approach. Juvenile salmon were counted within 50 m reaches starting at the tributary confluence and continuing upstream until no juvenile salmon were observed. The IP model predicted over 900 stream km in the basin to support high quality ($IP \geq 0.75$) rearing habitat. Occupancy estimation based on eDNA samples indicated that 80.2% (± 4.3 SE) of previously unsampled sites classified as high IP and 56.4% of previously unsampled sites classified as low IP were occupied. The probability of detection of Chinook Salmon DNA from three replicate water samples was high (0.76 ± 1.9 SE) but varied with drainage area. A power analysis indicated power to detect proportional changes in occupancy based on parameter values estimated from eDNA occupancy models. Results of snorkel surveys showed that the upper extent of juvenile Chinook Salmon within tributaries was from 200 to 1,350 m upstream of tributary confluences. Occurrence estimates based on eDNA and snorkel surveys generally agreed, but care should be taken to ensure that little temporal gap

exists between samples as juvenile salmon use of tributary habitats is likely often intermittent. Overall, the combination of IP habitat modeling, occupancy estimation based on eDNA, and snorkel surveys provided a useful, rapid-assessment method to predict and subsequently quantify the distribution of juvenile salmon in previously unsampled tributary habitats. These methods will provide tools for managers to rapidly and efficiently map critical rearing habitats and prioritize sampling efforts to expand the known distribution of juvenile salmon in interior Alaska streams.

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Introduction

Characterizing the distribution of fish species through time and across space is challenging, owing to variation in scale, life history, and logistics, and may be compounded by use of traditional sampling techniques that require large effort and have variable effectiveness (Hayes et al. 2012; Hubert et al. 2012; Comte and Grenouillet 2013). Distributions of anadromous species are particularly difficult because different life stages are often present in habitats for only a limited time. Newly developed rapid assessment methods may increase detectability when quantifying fish distributions, particularly for threatened or elusive species, and will be important to help identify critical habitats, which is important given anthropomorphic and climate change impacts (Dudgeon et al. 2006; Dawson et al. 2011; Baird and Hajibabaei 2012). However, development and application of such distributional rapid assessment methods, particularly in remote areas of interior Alaska, has not been implemented.

Historically, the Chena River has supported the second largest spawning population of Chinook Salmon in the U.S. portion of the Yukon River drainage, but recently returning adult abundance has declined (Eiler et al. 2006). An expert panel hypothesized changes to, or lack of, high quality rearing habitat that is conducive to growth and survival of juvenile Chinook Salmon as a potential driver of adult declines (Schindler et al. 2013). This offers a unique opportunity to both evaluate the utility of three types of rapid assessment methods to estimate quality rearing habitat and to better understand juvenile Chinook Salmon ecology and habitat use in the Chena River, Alaska.

Geographical information system (GIS)-based predictive models are useful tools to delineate the distribution of organisms across broad spatial extents. Derivation of digital maps of habitat from a digital elevation model (DEM) can allow for estimation of fish habitat potential and species distributions with minimal impacts to species (Agrawal et al. 2005; Burnett et al. 2007; Sheer et al. 2009; Bidlack et al. 2014). Such habitat potential models are based on suitability curves developed

using reach-scale relationships between fish presence or abundance and habitat characteristics. The model then pairs river geomorphic characteristics and suitability curves and assigns a continuous score from 0 to 1 (Burnett et al. 2007). The resulting score is indicative of rearing potential in the section of river and can be used to prioritize further sampling. Such models could be applied to a rapid assessment framework to identify potential critical habitats or focus sampling effort.

Environmental DNA (eDNA) assays are a rapid, non-invasive method to quickly assess presence/absence of organisms based on the detection of DNA molecules in environmental samples (Goldberg et al. 2011; Bohmann et al. 2014). In aquatic eDNA studies, DNA is extracted from filtered water samples. The isolated DNA can then be used in species-specific assays where gene sequences are targeted for detection and used to estimate presence of a species. This approach is particularly useful for endangered and elusive species for which traditional methods may be less effective (Goldberg et al. 2011). Environmental DNA likely derives from sloughing of skin and mucus, or excrement from cell shedding in the lining of the gut (Ficetola et al. 2008; Klymus et al. 2015). Although species-specific molecular assays required to detect aquatic organisms based on eDNA take some effort to develop, once optimized they provide a tool for detecting presence in other locations without having to be redeveloped (Laramie et al. 2015). Distribution data based on eDNA surveys can be used to corroborate model predictions or direct observations, or used as a “first pass” method to determine the presence of juvenile Chinook Salmon in a tributary watershed and prioritize more intensive sampling, all as part of a rapid assessment.

A direct-observation, aquatic bioassessment technique can be an additional useful tool for delineating distributions. For example, snorkeling offers a rapid, inexpensive, and non-invasive method to survey presence and abundance of fishes across expansive ranges in clear water systems (Armour et al. 1983; Thurow 1994; O’Neal 2007) because it does not require extensive equipment and can easily be used in remote locations. Additionally, snorkeling can be used in locations where

traditional techniques such as nets, traps, or electrofishing are unfeasible and with less effort and cost than capture-recapture or removal methods because it does not require handling potentially sensitive species. Moreover, both methods (eDNA and snorkeling) can be used to develop suitability curves for a region- or basin-specific habitat potential model.

The overall goal for this project was to develop and test the utility of a rapid assessment approach that combines a GIS-based habitat potential model, environmental DNA sampling, and snorkel surveys to delineate the distribution of juvenile Chinook Salmon rearing habitats in the Chena River basin, Alaska. Specific objectives were to (1) develop an intrinsic potential habitat model from the literature to predict the distribution of Chinook Salmon rearing habitats and aid with sample site prioritization for the Chena River, (2) use environmental DNA to assess presence/absence of juvenile Chinook Salmon among selected tributary habitats identified from objective 1, and (3) determine the spatial distribution of rearing juvenile Chinook Salmon within selected tributaries of the Chena River via snorkeling surveys. These methods will provide tools for managers to rapidly and efficiently map critical habitats and prioritize sampling efforts to expand the known distribution of juvenile salmon in interior Alaska streams.

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Chapter 1: A Rapid Assessment Method to Estimate the Distribution of Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in an Interior Alaska River Basin¹

ABSTRACT

Identification and protection of water bodies used by anadromous species in Alaska are critical in light of increasing threats to fish populations, yet challenging given budgetary and logistical limitations. Non-invasive, rapid assessment sampling techniques may reduce costs and effort while increasing species detection efficiencies. I used an intrinsic potential (IP) habitat model to identify high quality Chinook Salmon *Oncorhynchus tshawytscha* rearing habitats and select sites to sample throughout the Chena River basin for juvenile occupancy using environmental DNA (eDNA) and distribution within tributaries using snorkel surveys. Water samples were collected from 75 tributary sites in 2014 and 2015. The presence of Chinook Salmon DNA in water samples was assessed using a quantitative polymerase chain reaction (qPCR) assay targeting that species. Snorkel surveys were conducted and physical habitat was measured for a subset of tributaries examined with the eDNA approach. Juvenile salmon were counted within 50 m reaches starting at the tributary confluence and continuing upstream until no juvenile salmon were observed. The IP model predicted over 900 stream km in the basin to support high quality ($IP \geq 0.75$) rearing habitat. Occupancy estimation based on eDNA samples indicated that 80.2% (± 4.3 SE) of previously unsampled sites classified as high IP and 56.4% of previously unsampled sites classified as low IP were occupied. The probability of detection of Chinook Salmon DNA from three replicate water samples was high (0.76 ± 1.9 SE) but varied with drainage area. A power analysis indicated power to detect proportional changes in

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occupancy based on parameter values estimated from eDNA occupancy models. Results of snorkel surveys showed that the upper extent of juvenile Chinook Salmon within tributaries was from 200 to 1,350 m upstream of tributary confluences. Occurrence estimates based on eDNA and snorkel surveys generally agreed, but care should be taken to ensure that little temporal gap exists between samples as juvenile salmon use of tributary habitats is likely often intermittent. Overall, the combination of IP habitat modeling, occupancy estimation based on eDNA, and snorkel surveys provided a useful, rapid-assessment method to predict and subsequently quantify the distribution of juvenile salmon in previously unsampled tributary habitats. These methods will provide tools for managers to rapidly and efficiently map critical rearing habitats and prioritize sampling efforts to expand the known distribution of juvenile salmon in interior Alaska streams.

INTRODUCTION

Identifying and quantifying distributions of fish through time and across space is challenging, particularly when scale, life histories, ontogeny, and budgetary restrictions are considered (Franklin 2010; Comte and Grenouillet 2013), yet such activities are increasingly important given anthropomorphic impacts and recent and future climate change (Dudgeon et al. 2006; Dawson et al. 2011). However, use of traditional active fish capture techniques such as seining or electrofishing to quantify fish species distributions can be labor intensive and costly, and their effectiveness may vary across life stages (Hayes et al. 2012; Hubert et al. 2012). Moreover, these methods may be inappropriate for imperiled or sensitive species, especially if increased handling stress or mortality is an issue (Jerde et al. 2011). As a result, rapid assessment methods are needed that can address the challenges of using traditional methods to quantify distributions.

Mapping the distribution of anadromous species in freshwater ecosystems may add an additional element of difficulty because these species are often only present in habitats for a limited amount of time, depending on life stage. The Alaska Department of Fish and Game (ADFG) maintains a catalog that designates important aquatic pathways and habitats for various life stages of anadromous fishes in Alaska (Anadromous Waters Catalog [AWC]; State of Alaska 2016). It is estimated that more than 20,000 rivers or lakes with the potential to support anadromous species remain undesignated by the AWC owing to lack of sampling and logistical challenges in this remote Arctic environment (State of Alaska 2016). The case of the AWC highlights challenges inherent to mapping fish distributions, especially in Alaska.

Newly developed rapid assessment methods may increase efficacy when quantifying fish distributions, particularly for threatened or elusive species, and will be important to help identify critical habitats in a changing environment (Dawson et al. 2011; Baird and Hajibabaei 2012). Such methods have been used to successfully satisfy a variety of objectives in aquatic research and monitoring, including classification of physical habitat characteristics (Nadeau et al. 2015), assessment of aquatic toxicity (Bulich et al. 1981), and management of invasive species (Leung et al. 2005). Rapid aquatic bioassessment techniques can reduce cost and effort while increasing detectability (i.e., the probability of observing an individual, given its presence) while being non-invasive, which is important for sensitive species (Jerde et al. 2011). However, if not accounted for, imperfect detection can bias estimates of habitat use, relative abundance, and the effects of predictor variables in models of fish-habitat relationships (Tyre et al. 2003).

Predictive models developed in a geographical information system (GIS) framework are useful tools to predict the spatial distribution of fish and their habitats while minimizing impacts on sensitive species, logistical challenges resulting from limited site access, and imperfect

detection. Such models allow for estimation of fish habitat potential, delineate species distributions, or guide restoration efforts without laborious, on-the-ground sampling, provided the necessary spatial data (e.g., digital elevation models; DEM) are available with which to parameterize models (Burnett et al. 2007). Effort and costs required are minimal, but such models can produce continuous estimates of habitat potential at relatively fine spatial scales (e.g., 50-100 m stream reaches). One such approach, termed *intrinsic habitat potential*, has been used to predict Coho Salmon (*Oncorhynchus kisutch*) and Steelhead (*O. mykiss*) rearing habitat potential in Northern Oregon river systems (Agrawal et al. 2005; Burnett et al. 2007), and Chinook Salmon (*O. tshawytscha*) rearing potential in the Columbia and Copper River basins (Agrawal et al. 2005; Sheer et al. 2009; Bidlack et al. 2014). However, in regions such as Alaska where spatial data are lacking, implementation of habitat potential models remains challenging, and to date such models are rarely implemented (see Bidlack et al. 2014).

Environmental DNA (eDNA) assays are a rapid, non-invasive method to quickly assess presence/absence of aquatic organisms and is particularly useful for detecting endangered, invasive, and elusive species in stream systems (Goldberg et al. 2011; Bohmann et al. 2014). It is believed that target DNA originates from sloughing of cells and tissues (Ficetola et al. 2008). Species-specific DNA can be detected in a number of materials including water, feces, and sediment. However, detection can be influenced by the amount of DNA in the system (e.g., density, dilution, diffusion, etc.), as well as factors that affect its viability such as water temperature and sun exposure (Bohmann et al. 2014; Merkes et al. 2014). In eDNA assays designed to detect a particular species, the pool of DNA in an environmental sample is isolated and used in species-specific PCR-based copying of short mitochondrial DNA sequences. The

production of PCR products indicates presence of target species (Goldberg et al. 2011; Jerde et al. 2011).

Development, validation, and optimization of species-specific eDNA assays can be laborious and consume substantial resources. However, once an assay is validated and optimized, it is relatively inexpensive to implement particularly with larger sample sizes. To date, most aquatic eDNA research has focused on methodological issues, but increasing effort has been given to monitor species distributions and community composition (Bronnenhuber and Wilson 2013; Takahara et al. 2013) using eDNA for a number of fishes including Bighead (*Hypophthalmichthys nobilis*), Silver (*H. molitrix*), and Common Carp (*Cyprinus carpio*; Klymus et al. 2015; Barnes et al. 2014; Takahara et al. 2012, 2015; Turner et al. 2015; Jerde et al. 2011; Merkes et al. 2014; Mahon et al. 2013), Brook Trout (*Salvelinus fontinalis*; Wilcox et al. 2013; Jane et al. 2015), Siberian Sturgeon (*Acipenser baerii*; Dejean et al. 2011), and European Weather Loach (*Misgurnus fossilis*; Sigsgaard et al. 2015).

Observational sampling methods, such as snorkel surveys, offer additional rapid and non-invasive method options to survey presence and abundance of fishes across expansive ranges in clear-water systems (Armour et al. 1983; Thurow 1994; O'Neal 2007). Snorkeling can be employed in remote locations where use of nets, traps, or electrofishing is impracticable, and with less effort and cost than capture-recapture or removal methods. However, direct observation methods such as snorkel surveys are only useful if their results are accurate and precise. For example, snorkel estimates may be biased owing to differences among observers, water clarity, habitat complexity, or other factors (Hillman et al. 1992; Rosenberger and Dunham 2005).

Our overall goal was to develop and test the efficacy of a rapid assessment approach that combines GIS-based habitat potential modeling, eDNA sampling, and snorkel surveys to delineate the distribution of juvenile Chinook Salmon rearing habitats in an interior Alaska river-system. Our specific objectives were to (1) develop a predictive habitat model to continuously estimate Chinook Salmon rearing habitats across an interior Alaska river basin, (2) use eDNA and occupancy estimation to assess presence of juvenile Chinook Salmon in tributaries, and (3) conduct snorkel surveys within these same tributaries to corroborate eDNA estimates and delineate the spatial distributions (i.e., upstream extent) of juvenile salmon. Here we evaluate the utility and accuracy of these methods to provide managers with an efficient and inexpensive approach to identify and prioritize critical rearing habitats, which will lead to a better understanding of juvenile salmon ecology and improve evaluation of population vital rates and conservation status.

METHODS

Study area.— The Chena River (watershed area ~ 5,300 km²) is a clear-water tributary of the Tanana River, located in the Yukon River basin near Fairbanks, Alaska (Figure 1.1). The Chena River basin has five major tributaries that provide flow to the main stem (North, South, West, and East Forks, and the Little Chena River), with stream length within the Chena River network totaling approximately 2,300 stream-km. Stream flow in the Chena River basin originates from precipitation, snowmelt, and groundwater (Bennett et al. 2015). The Chena River hosts a diversity of aquatic habitats ranging from small- and medium-sized creeks with pool-riffle-run complexes, to larger river habitats with large pools, and numerous sloughs, backwaters, and interconnected ponds. These habitats support a relatively diverse fish assemblage including: Arctic Lamprey (*Lethenteron camtschaticum*), Alaska Brook Lamprey (*L. alaskense*), Longnose

Sucker (*Catostomus catostomus*), Slimy Sculpin (*Cottus cognatus*), Northern Pike (*Esox lucius*), four species of whitefish, Inconnu (*Stenodus leucichthys*), Burbot (*Lota lota*), Lake Chub (*Couesius plumbeus*), Arctic Grayling (*Thymallus arcticus*), Chum Salmon (*Oncorhynchus keta*), Coho Salmon (*O. nerka*), and Chinook Salmon (*O. tshawytscha*; State of Alaska 2016).

Historically, the Chena River has supported one of the largest Chinook Salmon spawning populations in the Yukon River basin but, similar to the rest of the basin, has recently seen significant declines in adult returns (Eiler et al. 2006; ADF&G 2013; Schindler et al. 2013). From 1986-2009, adult escapement averaged 6,400 fish, but for 2010-2013, estimates averaged less than 2,000 individuals (Savereide and Huang 2014). The dominant life-history type of Chinook Salmon in interior Alaska is the “stream-type life history” with juveniles spending a full year rearing in freshwater before migrating to the ocean and 1 to 7 years at sea before returning as mature adults to natal streams to spawn in late summer and fall (Healey 1991). Chinook Salmon spawn predominately in the main stem of the Chena River from river km (rkm) 36 to 179 and to a lesser extent in the South Fork and Middle Fork tributaries (State of Alaska 2016; Figure 1.1). Chinook Salmon embryos and alevins remain in the gravel until early spring when fry emerge to initiate feeding. It is generally understood that fry disperse from redds via passive or directed movements and enter rearing habitats to feed and grow (Copeland et al. 2014). In the Chena River basin, juveniles are known to rear in main stem habitats, often in the presence of accumulations of large woody debris (i.e., logjams; Perry 2012; Neuswanger et al. 2014; Wipfli et al. 2014). However, evidence suggests that small tributaries (e.g., > 20 km² contributing area; State of Alaska 2016) and main stem off-channel habitats (B. Huntsman, UAF *unpublished data*) are also used extensively. Because there is incomplete information for the Chena River basin on

the extent of small tributary use, we chose to focus on those areas, while expecting juveniles to be present in tributaries with large woody debris (State of Alaska 2016; Neuswanger et al. 2014).

Juvenile Chinook Salmon habitat potential.—We used an intrinsic potential (IP; Burnett et al. 2007) approach to estimate the potential for high-quality juvenile Chinook Salmon rearing habitat throughout the Chena River basin (including main stem and tributary habitats) and to inform sample site selection for eDNA and snorkel surveys conducted in tributaries (see below). Intrinsic potential is based on the relationship between juvenile Chinook Salmon habitat use and relevant geomorphic and hydrologic stream attributes (e.g., gradient, valley confinement, mean annual flow). These physical attributes were derived using a digital landscape model parameterized for the Chena River basin (NetMap; Benda et al. 2007). The NetMap model generates a synthetic digital stream network layer from a 5-m resolution digital elevation model (DEM) based on flow accumulation and channel delineation algorithms (described in Clarke et al. 2008). The result is a network of 50-200 m stream reaches linked to the surrounding landscape and attributed with geomorphic characteristics (e.g., gradient, stream width, drainage area, etc.).

We selected three attributes previously used for juvenile Chinook Salmon to build our IP model (Sheer et al. 2009; Bidlack et al. 2014). The first was gradient (%), GRAD), which was generated by Netmap based on the DEM and the synthetic stream network (Clarke et al. 2008). Gradient can act as a physical barrier by creating high velocity reaches of river that would make it difficult for juveniles to maintain position in the water column because of high energy expenditure (Raleigh et al. 1986; Sheer et al. 2009). The second attribute was mean annual discharge ($\text{m}^3 \cdot \text{s}^{-1}$, MAD). We considered this factor to be a proxy for stream size because discharge typically increases with stream size (Clarke et al. 2008). Adult Chinook Salmon spawn

in larger rivers in the presence of suitably sized substrate, and these habitats are often located in close proximity to high quality juvenile rearing habitats (Falke et al. 2013). Moreover, large streams with high discharge provide increased feeding opportunities for juvenile salmon via mass transport of invertebrate food resources. In relatively unaltered systems such as those in interior Alaska, large rivers contain complex habitats such as accumulations of large woody debris, which form areas of low current velocities that serve as flow refugia (Neuswanger et al. 2015). The final attribute was valley constraint (ratio of bank full- to valley-width, VAL), a measure of the extent to which the stream interacts with the floodplain (Burnett et al. 2007). In relatively pristine environments, unconstrained reaches have high habitat complexity through the presence of backwater and off-channel habitats and accumulation of large woody debris (Montgomery and Buffington 1997, 1998).

Burnett et al. (2007) suggested that IP models should be developed using at least three suitability curves constructed from empirical data and/or expert opinion. Suitability curves are a measure of juvenile fish habitat use across a range of habitat conditions. They consist of index scores (0-1) assigned across the range of habitat values independently for each geomorphic or hydrologic attribute (Figure 1.2). Because index scores were not available for juvenile Chinook Salmon in the Chena River, we synthesized three previously developed juvenile Chinook Salmon IP models that included the Copper River, Alaska (Bidlack et al. 2014), and rivers in Northern California and Oregon (Sheer et al. 2009). The curves for the aforementioned studies were developed using empirical data and expert opinion. For each of the three models and attributes, we averaged index scores across the range of provided values to produce the suitability curves

used in this study. Then using our curves, we calculated IP scores for individual stream reaches as follows:

$$IP = (GRAD \times MAD \times VAL)^{1/3} \quad \text{Eq. 1}$$

where GRAD, MAD, and VAL are index scores derived from the respective suitability curves for attribute values calculated for a particular reach. The IP scores were applied to stream reaches throughout the Chena River basin using the Netmap extension (Benda et al. 2007) for ArcGIS version 10.2.1 (ESRI 2011).

Sample site selection.—We used IP scores in combination with available data on the known rearing distribution of juvenile Chinook Salmon in the Chena River basin (State of Alaska 2016) to select tributaries to sample for fish presence using eDNA and distribution based on snorkel surveys. First, we divided the Chena River basin into 149 tributary catchments with contributing areas $> 20 \text{ km}^2$. Next, we categorized each catchment as being within the known distribution of juvenile Chinook Salmon in the Chena River basin or not based on the AWC. Catchments outside of the known distribution were classified using results of the IP scoring as those that contained high quality juvenile Chinook Salmon rearing habitat potential ($IP \geq 0.75$), and those that did not. The high/low cutoff (0.75) has been used in previous studies (Bidlack et al. 2014). Our schema resulted in three categories: low IP, high IP, and known rearing (AWC). Ten catchments from each of the three categories were randomly selected to sample fish presence and distribution during the 2014 and 2015 seasons.

Environmental DNA field methods.— Our field methods for eDNA collection followed those of Pilliod et al. (2012). We elected to filter samples in the laboratory rather than the field to reduce the risk of cross contamination between sites. Sample collection bottles were sterilized in a 50% bleach solution, rinsed with deionized water, and allowed to dry prior to collection. Once

dry, lids remained tightly closed and unopened until sampling occurred. At each site, three sterile 1-L bottles used for water collection were rinsed in river water three times and then filled in the stream current near the water surface. Samples were stored in a cooler with ice and returned to the lab for filtration. Water samples were collected at tributary confluences about 10 m upstream of the main stem mixing area to avoid potential contamination from water in the main river channel.

Environmental DNA laboratory methods.—All laboratory equipment was sterilized with a 50% bleach solution, rinsed with deionized water, and allowed to dry between samples to prevent cross-contamination and false-positives. A 1-L sample of water was filtered through a 47 mm, 0.45- μ m cellulose nitrate filter (Whatman International Ltd., Little Chalfont, United Kingdom). Using sterile forceps, the filters were folded into quarters, rolled, and placed in a vial with \geq 98% ethanol. Samples from the 2015 sampling season were cut in half; one half was archived and the other used for DNA extraction.

The DNA was extracted from the filters using a modified phenol-chloroform-isoamyl alcohol (PCI) DNA extraction protocol described in Renshaw et al. (2014). Briefly, the ethanol in which filters were stored was decanted and replaced with 800 μ L of lysis buffer to increase extraction efficiency by lysing cells and releasing DNA. Filters were then incubated at 65°C for 30 minutes prior to the addition of 800 μ L of PCI (one phase, 25:24:1; Amresco LLC, Cleveland, Ohio). The vial was centrifuged at 15,000 g for 5 minutes and 600 μ L of DNA-containing aqueous phase was carefully transferred to a clean 1.5 ml micro-centrifuge tube. Chloroform-isoamyl alcohol (24:1, Amresco LLC, Cleveland, Ohio) was added (600 μ L) and the vial centrifuged at 15,000 g for 5 minutes. This time, 400 μ L of the aqueous layer was transferred to a new tube. One mL of 100% cold ethanol and 16 μ L of 5 M NaCl was added to the tubes and

the DNA was allowed to precipitate overnight at -20°C. The resulting precipitate was pelleted by centrifuge for 10 minutes at 15,000 g and dried until no visible liquid remained. Pellets were dissolved in 50 µL of low EDTA TE buffer (10 mM Tris, 0.1 mM EDTA) and frozen until real-time quantitative polymerase chain reaction (qPCR) analysis was performed.

We used a qPCR protocol that relied on species-specific PCR primers and a minor groove binding (MGB) probe that binds specifically to the resulting PCR amplicons (Takahara et al. 2012). We used a previously developed Chinook Salmon-specific eDNA assay under the following cycling parameters: hot start 95°C for 15 minutes followed by 55 cycles at 94°C for 60 s and 60 °C for 60 s (Laramie et al. 2015). Laramie et al. (2015) states that their primer is optimized for 70°C, but we had trouble with amplification using that temperature for the annealing and extension step. The rest of the reagents are optimized for annealing and extension at 60°C, and we had success running our samples using that temperature.

We used optical quality 96-well plates and seals. Each well contained Quantitect MasterMix (Qiagen, Inc., Hilden, Germany), Chinook Salmon primers, MGB probe, and Taq-Man exogenous internal positive control reagents (Life Technologies, Corporation, Carlsbad, California). The Quantitect Mastermix contains all PCR reagents with the exception of oligonucleotides and template DNA. For a single reaction totaling 15 µL, we used 7.5 µL of 1X Quantitect Mastermix, 0.6 µL 0.4X of EXO-IPC Mix, 0.3 µL of 1X EXO-IPC DNA, and 0.75 µL of a 1X primer/probe mix working stock. The exogenous internal positive control (EXO-IPC) is a self-contained qPCR assay which serves as a control to detect presence of PCR inhibitors in our DNA preparations. Amplification failures of the EXO-IPC product indicate presence of PCR inhibitors in the assembled reaction. The presence of PCR inhibitors can lead to false negative results. However, if there is no target species detection in the samples but the EXO-IPC

produces the expected amplification, then it is likely that there is either no target DNA or it is present at concentrations lower than the assay's sensitivity threshold.

Because we were unable to amplify the filtered eDNA preparations, we diluted the samples 100-fold in molecular biology grade water. The dilution allowed us to reduce the concentration of qPCR inhibitors in the sample to low enough levels that the DNA could be amplified. All samples were tested in triplicate reactions alongside two or more sets of serial dilutions (10^{-2} - 10^{-6}) of positive control DNA preparations, three negative controls (PCR grade water), and three positive controls (large known amounts of Chinook Salmon DNA).

Snorkel surveys.—We conducted snorkel surveys to compare with occurrence estimates from the eDNA surveys and to estimate the distribution (i.e., upstream extent) of juvenile Chinook Salmon in Chena River basin tributary habitats. We used snorkeling instead of active capture techniques owing to sampling permit stipulations aimed to reduce stress and mortality for this sensitive life stage but also because of reduced cost and effort of snorkeling. Each tributary was divided longitudinally into a set of 50-m reaches beginning at the tributary confluence. Snorkel surveys were initiated in the first reach adjacent to the tributary and repeated every 150 m until no juvenile Chinook Salmon were observed in three consecutive sample reaches (Thurow et al. 2012). Within each selected reach, the snorkeler moved upstream in a zig zag pattern across the stream and all juvenile Chinook Salmon were identified and counted. In streams where the velocity was too high for upstream movement, the snorkeler would float downstream on both sides of the creek to ensure that full coverage of the stream was reached.

Reach-scale habitat surveys.—Habitat measurements were taken during each snorkeling survey to investigate the relationship between juvenile Chinook Salmon presence or abundance and tributary habitat characteristics. Habitat measurements included mean channel width (m),

maximum depth (cm), dominant substrate, water clarity (scale of 1-5), water temperature (°C), weather, and percent coverage of large woody debris (Thurow 1994). Channel width was measured via transects located at 0, 25, and 50 m along each 50-m sample unit. Clarity was measured on a scale of 1-5 and determined by snorkeler, with 1 being crystal clear, 2 being tannic and dark water, 3 being turbid, which required snorkeling on both sides of the creek, 4 being both turbid and tannic, and 5 being unsnorkelable. Water temperature was measured at the surface. Dominant substrate was identified visually and categorized by type: silt, sand, gravel, cobble, or boulder (Buffington and Montgomery 1999).

Data analysis.— We fitted single-season occupancy models (MacKenzie et al. 2003) to detection/non-detection data from eDNA surveys to evaluate factors likely to influence the presence of juvenile Chinook Salmon in Chena River tributary habitats. This approach allowed joint estimation of detectability and the proportion of sites occupied using the occupancy estimation framework established by MacKenzie et al. (2006). The framework uses replicate samples to incorporate the influence of non-detection and habitat covariates on estimates of the proportion of sites occupied. We determined the best model, given the data, using a two-stage approach where we first fitted occupancy models with only detection covariates, and based on those results, subsequently fitted occupancy models using habitat covariates that included the detectability covariate(s) identified in stage one. All occupancy analyses were conducted using package “unmarked” (Fiske and Chandler 2011) in program R (R Core Team 2015).

Covariates hypothesized to influence detectability included mean July through September summer flow (summer flow; m^3/s) and drainage area (km^2). We selected the summer flow metric to capture the large observed difference in discharge between 2014 and 2015. During 2014, the Chena River experienced record high water and had five flood events between June and August,

which could have reduced detectability as a result of dilution of DNA in the water. Tributary- and year-specific flow metrics were calculated using a variable infiltration capacity rainfall-runoff model for the Chena River basin (Bennett et al. 2015; Huntsman et al. *UAF unpublished data*). Because model predictions were only available for 1970-2010, we identified years within that period with summer flows similar to 2014 and 2015. We selected drainage area as a detection covariate because increased drainage area might result in lower detectability because there is more water to dilute DNA in the tributary. Drainage area for each tributary was estimated using ArcGIS. We used Akaike's information criterion (AIC) to select the model with the best fit, given the data. We considered the best model as that which had the lowest AIC value and the highest model weight (w_i).

We tested whether the proportion of sites occupied varied by sample year (2014, 2015), category (High IP, Low IP, AWC), summer flow (m^3/s), and drainage area (km^2). Sample year and summer flow were included to test for differences in the proportion of sites occupied in a high flow year (2014) versus an average flow year (2015). Drainage area was included to evaluate if occupancy varied with tributary size. Finally, we included category to test the efficacy of the IP model to predict tributaries occupied by juvenile Chinook Salmon. The best model, given the data, was determined using AIC model selection as detailed above.

Because we were interested in evaluating the utility of eDNA sampling as a monitoring tool, we conducted a power analysis to assess our ability to detect proportional changes in occupancy (R) while varying detection probability (p), sample replicates (K), and the number of sites sampled (S) at given levels of occupancy (ψ) based on methods proposed by Guillera-Aroita and Lahoz-Monfort (2012). The range of values for these parameters was based on the results of our occupancy analysis.

RESULTS

Juvenile Chinook Salmon habitat potential.—Our IP model predicted that of approximately 2,265 total stream-km in the Chena River basin, 931 km had an IP score of ≥ 0.75 indicating high juvenile Chinook Salmon rearing habitat potential in these stream reaches (Figure 1.3). High IP reaches were concentrated along the main stem (196 stream km; Table 1.1), but also occurred in tributaries. The sub-basin with the largest proportion of high IP stream reaches was the Lower main stem (29%), but the South Fork (23.5%) and the Little Chena River (21.6%) were also predicted to contain abundant high quality juvenile salmon rearing habitat. In contrast, the North Fork and West Fork sub-basins had the lowest proportion of high IP reaches at 1% and 3%, respectively. Across the basin for all catchments $> 20 \text{ km}^2$ drainage area, 86 catchments were categorized as high IP (≥ 0.75), 31 as low IP (≤ 0.75), and the remaining 32 catchments were known rearing habitat as designated by the AWC, totaling 149 catchments.

Environmental DNA.—We sampled 35 tributaries for eDNA in 2014 and 40 tributaries during 2015. Twenty-six tributaries were sampled in both years. Raw estimates (i.e., uncorrected for imperfect detection) indicated that juvenile Chinook Salmon were detected in 70% of tributaries and in 60% of samples (Table 1.2). In 2014, Chinook Salmon were detected in 16 of 35 (46%) tributaries, whereas fish were detected in 29 of 40 (73%) in 2015.

The negative and positive controls performed as expected. We found no amplification in any of the negative controls indicating consistency and a lack of false positives. The positive controls and serial dilutions also amplified as expected.

Across sites, Chinook Salmon DNA was not detected in any of the replicate samples at 40% of sites (i.e., detection history $[h_i] = 000$), whereas for 31% of the sites DNA was detected in all of the replicate samples ($[h_i] = 111$). The remaining sites had either one or two detections

(12%: $[h_i] = 100$, 17%: $[h_i] = 110$). The best model for detectability, given the data, indicated that p increased with drainage area (Table 1.3; Figure 1.4). Overall, p was high (0.76 ± 0.02), indicating that our sampling methods had a high probability of detecting Chinook Salmon DNA, if the species was present.

The proportion of sampled tributaries occupied by juvenile Chinook Salmon in the Chena River basin was estimated to be 0.61 ± 0.03 . The top occupancy model contained almost half the weight of all candidate models, and included only the category covariate (AIC = 245.06, $w_i = 0.49$), even though there was some evidence for models that included summer flow, drainage area, and to a lesser extent year (Table 1.3). Juvenile salmon were estimated to occur in 0.80 ± 0.10 of sites with high rearing habitat potential (high IP; Figure 1.5). Interestingly, juvenile Chinook Salmon were detected at about half (AWC = 0.47 ± 0.10 , low IP = 0.56 ± 0.15) of the sites where rearing habitat potential was predicted to be lower (e.g., < 0.75) or this life stage had been observed to be present historically.

Based on our power analysis, the power to detect proportional changes in occupancy (R) increased with S (Figure 1.6a). Conversely, we did not see an increase in power with an increase in K and fewer replicates resulted in only slightly less power to detect changes than the reference line (Figure 1.6b). Similarly, R did not increase when p increased over our value of 0.76, but our ability to detect changes in occupancy under scenarios of lower p was much less (Figure 1.6c). Power increased and decreased with variation in the proportion of sites occupied (Figure 1.6d). Interestingly, power curves were not symmetrical around zero, indicating greater power to detect positive relative to negative proportional changes in occupancy.

Snorkel surveys.—Snorkel surveys conducted in 2014 were largely unsuccessful owing to high water levels and low water clarity. We snorkeled 10 tributaries, and only four juvenile

Chinook Salmon were observed in a single tributary. Those individuals were located 200 m upstream from the confluence with the main stem. Comparatively, we snorkeled 15 tributaries in 2015, and juvenile Chinook Salmon were observed and enumerated at six of the 15 sites (Figure 1.3). At sites where fish were present, density ranged from 0.009 fish·m⁻¹ in the West Fork of the Chena River to 0.859 fish·m⁻¹ in Rock Creek. The upstream extent of juvenile salmon use of tributaries ranged from 200 to 1,350 m from tributary confluences (Table 1.4). Juvenile Chinook Salmon were found in tributaries with a variety of habitat characteristics. The most common dominant substrates were gravel and cobble, but fish were also present when finer substrates (e.g., mud and sand) dominated. Juvenile Chinook Salmon were found in streams with instantaneous temperature readings that ranged from 4.6°C up to 11.1°C. The mean width of the tributaries was 4.93 m (± 3.0 m SD). Of the six sites where juvenile salmon were observed, four sites were categorized as AWC and two were low IP.

Comparison of snorkel surveys and eDNA.—We compared results from the 15 sites where both eDNA sampling and snorkel surveys were conducted during the same year using a confusion matrix (Table 1.5). Results from the two techniques agreed for nine of 15 sites (detected = 4, not detected = 5). At two sites, we observed juvenile Chinook Salmon using snorkeling but DNA was not detected, and at four sites DNA was detected but fish were not observed.

DISCUSSION

The combination of intrinsic potential habitat modeling, occupancy estimation based on eDNA, and snorkel surveys provided a useful, rapid-assessment method to predict and subsequently quantify the distribution of juvenile salmon in previously unsampled tributary habitats in an interior Alaska river system. Based on this assessment, we determined that juvenile

Chinook Salmon were heavily using tributary habitats to rear and were able to add to our knowledge of their distribution in the Chena River basin. Such rapid-assessment methods are critical in light of increasing threats to fish populations and necessary given difficulties associated with assessing basin-wide distributions. The case of the Chena River basin and other interior Alaska rivers is not unique; the methods we have developed will be useful in other situations where distribution data are needed but budgetary or logistical issues preclude rigorous broad-scale sampling efforts. Although the three methods have limited utility alone, in combination they provide a powerful tool to estimate fish distributions in stream networks. Below we discuss the advantages and disadvantages for each method alone and in combination.

Intrinsic potential.—The intrinsic potential GIS habitat model was useful to (1) predict juvenile Chinook Salmon rearing habitat suitability across a large, complex stream network at a relatively fine spatial scale (e.g., 50 – 200 m stream reaches), and (2) provide the basis for a sampling design to estimate occupancy of juvenile salmon in tributary habitats. A drawback of this approach is that it required detailed spatial data as well as knowledge of the relationships between juvenile salmon abundance and geomorphic habitat attributes. For example, our model required three reach-scale geomorphic attributes paired to the stream network, which were derived from a DEM. Gradient and mean annual flow are easily computed from precipitation data, a DEM and digital stream network using basic GIS analysis tools (Brabets 1996; Isaak et al. 1999). Valley constraint is more complicated, but efforts are being made to improve calculation of this metric (HSC 2011). Although such information is readily available via public databases for the contiguous United States (HSC 2011; EPA 2015), even basic aquatic spatial data for the state of Alaska is lacking. Acquisition of the relevant spatial data is mostly free provided the stream network has been mapped. Spatial data remains challenging for remote areas such as

interior Alaska, but is clearly important given the utility of recently derived GIS-based habitat models such as ours and Bidlack et al. (2014). Once the spatial data is obtained, given some GIS and NetMap expertise, the model can be applied to large extents with minimal effort.

The IP model is based on the relationship between fish abundance and geomorphic attributes of streams assumed to be associated with high quality rearing habitat. The IP approach has been successfully used to predict habitat potential for juvenile and adult salmonids, thus the approach can be tailored for specific life stages (Burnett et al. 2007; Sheer et al. 2009; Busch et al. 2011; Bidlack et al. 2014). The ability to modify the suitability curves for a particular species or life stage is advantageous because habitat suitability relationships vary among species and across life stages. However, the model requires existing knowledge, whether from empirical data or expert opinion, that can be used to establish the suitability curves (Burnett et al. 2007). There is error associated with constructing suitability curves such as inaccurate representation of habitat use from empirical data or disagreement between models built for the same species based on expert opinion. We chose to average three sets of juvenile Chinook Salmon suitability curves to reduce this error. However, region-specific parameterization of index curves is likely the best method to ensure accurate and precise habitat potential estimates.

Our IP model was based on three habitat attributes that have been useful in other river basins in the Pacific Northwest and Alaska to characterize juvenile Chinook Salmon rearing habitat potential. Although the systems where juvenile Chinook Salmon rearing IP models previously have been implemented differ widely in physical attributes, one factor common to all was the presence of rearing Chinook Salmon. However, the IP approach is flexible and various alternate attributes could be used if appropriate (e.g., glacial coverage; Bidlack et al. 2014). For example, in the Chena River basin and other rivers in interior Alaska, large woody

debris is known to provide critical rearing habitat for juvenile Chinook Salmon (Perry 2012; Neuswanger et al. 2014). Use of a proxy attribute such as sinuosity to represent the potential for wood accumulation might be valuable in these systems, or preferably a direct prediction of reach-scale wood recruitment (sensu Flitcroft et al. 2016). However, predicting wood recruitment requires additional spatial data such as forest species composition and structural characteristics (e.g., LEMMA; <http://lemma.forestry.oregonstate.edu/>) that may not be available for all areas. Overall, we found the IP habitat potential approach a useful, inexpensive, and non-invasive method to estimate the distribution of juvenile Chinook Salmon, and suggest it has potential to be a valuable tool that can be parameterized for other species and life stages.

Occupancy estimation—Environmental DNA combined with the occupancy analysis allowed us to ground truth the IP model predictions and provided an unbiased estimate of the proportion of tributaries used by juvenile Chinook Salmon in the Chena River basin. Drainage area had the largest effect on detection probability relative to other predictors (e.g., summer flow). This result was unexpected because the Chena River experienced record high water caused by several consecutive flood events during 2014. We anticipated that high water would reduce detection because diffusion and dilution can play a role in detection of target DNA (Bohmann et al. 2014). Unexpectedly, we found that our detection probability increased with drainage area, although there was considerable uncertainty surrounding this estimate. A potential explanation is that larger tributaries have a greater capacity for rearing juveniles and therefore might have a higher density of DNA relative to smaller tributaries with fewer fish.

When we considered occupancy as a function of covariates, the top model included only the habitat intrinsic potential category (low IP, high IP, AWC). We expected that year or summer flow would be important variables owing to flood events. Although not included in the top

model, there was modest support for the importance of summer flow ($w_i < 0.18$). Raw data results suggested lower occupancy in 2014 (high flow year) versus 2015 (moderate flow year) where Chinook Salmon DNA was detected in 46% and 73% of sites, respectively. Differences in tributary occupancy between the two years could have resulted from (1) juveniles remaining in the main stem or moving lower in the basin to rear during the high flow year, (2) juvenile recruitment and subsequent abundance being reduced owing to high flow conditions (Neuswanger et al. 2014), and/or (3) reduced detectability because of DNA being diluted or diffused owing to high flows. Further, research on the relationship between tributary habitat use by juvenile Chinook Salmon and flow conditions is warranted.

The cut-off value for high IP (e.g., > 0.75) that we selected might have been too conservative for our system. Seven tributary catchments that were categorized as low IP and found to be occupied fell within 0.15 of the cut-off. Because of this, the low IP rank might not be indicative of low rearing potential but may be a factor of how we chose to categorize habitat potential. Choice of a cut-off value could impact estimated occupancy values. For example, if several of the low IP tributaries have an IP score of 0.72 and the target species is detected, then the occupancy estimation for the low IP tributaries might be falsely inflated. Quantifying the relationship between IP score and occupancy would allow for estimation of a more relevant and appropriate threshold for the target species but such analysis is beyond the scope of this work.

The proportion of AWC sites that were occupied was lower than expected. This could be because some of the data incorporated into the AWC were over 10 years old (State of Alaska 2016). Species misidentification or a single juvenile observed in a stream could warrant the tributary being classified as a known rearing location because photos are not required for confirmation (State of Alaska 2016). Additionally, the AWC does not include reach-scale point

data but presence-only range data including the upper and lower extents (State of Alaska 2016). Presence-only data are subject to strong temporal and spatial variability and errors (Ottaviani et al. 2004). Given the natural and anthropomorphic changes that have occurred in the Chena River basin (TVWA 2015), it is possible that rearing habitat for juvenile Chinook Salmon may be less suitable today or that tributaries are being used by juveniles intermittently throughout their freshwater stage and the AWC data was unable to capture that movement.

Environmental DNA— While environmental DNA has proven to be a useful and effective method in stream habitats, there are advantages and limitations of the eDNA technique to consider when interpreting the results and/or designing a study. For example, eDNA methods cannot distinguish between DNA originating from live versus dead fish (Merkes et al. 2014). In our case, the source of detected DNA could be an adult Chinook salmon, whether alive or dead, or fecal matter from a number of terrestrial organisms that ingested Chinook Salmon DNA (Bohmann et al. 2014). As a result, unlike the IP and snorkeling approaches, eDNA cannot differentiate between life stages of a species. We addressed this issue through our study design; we only sampled habitats where juveniles but not adults were expected to be present. Additionally, although qPCR analysis can be used to quantify the amount of DNA present in a sample and thus provide an estimate of relative abundance of individuals (Takahara et al. 2012), owing to the presence of inhibitors and lack of 100% efficiency in our assays we were unable to take advantage of this approach.

Because collection effort is low and samples can be taken quickly, eDNA can be used for assessments of large spatial extents without impacting fish. Field collection requires very little training, and the lab work required to process samples is also quick provided the facilities are available, the eDNA assay have been developed, and the processes are optimized. As the eDNA

technique is used more widely and the number of validated assays increases, sample processing and analysis will decrease in cost (Ficetola et al. 2008).

The utility of environmental DNA as an assessment or monitoring tool depends on the objectives of the study. For example, eDNA would not be an appropriate tool to use for a distribution analysis in main stem stream reaches where multiple life stages co-occur because eDNA cannot differentiate among life stages. However, in main stem reaches, if the goal was to determine species presence regardless of life stage, for example to prioritize more intensive sampling or to determine if an endangered or extirpated species was present, then eDNA will be quite useful (Thomsen and Willerslev 2015). Environmental DNA analysis has been used to document the arrival of migratory anadromous Chinook Salmon into fresh water based on repeated sampling (Laramie et al. 2015), but utility of the technique for highly mobile resident species is likely limited due to the persistence of DNA in a system as well as the ubiquity of the species (Merkes et al. 2014; Goldberg et al. 2015).

Snorkel surveys— Conducting snorkel surveys proved to be more difficult than initially expected as water clarity limited our ability to successfully sample all tributary habitats during both years. Due to record precipitation and flow levels in 2014, we were unable to access several tributaries due to the high water and unsafe river conditions. The high water also greatly decreased water clarity in many tributaries, which precluded snorkeling. In 2015, the Chena River did not receive as much precipitation, water levels remained low, and clarity was high throughout the majority of the sampling period. However, even in this lower flow year a number of tributaries had poor water clarity owing to other factors such as high sediment loading or the presence of tannins, which stained the water tea-colored and decreased visibility. Regardless,

under the right conditions we found snorkel surveys were a useful, non-invasive method to determine the upstream extent of tributary use by juvenile salmon.

Occurrence estimates based on eDNA and snorkel surveys during 2015 generally agreed, with most (nine of 15) sites matching. Exceptions included two sites where snorkelers observed juvenile Chinook Salmon but DNA was not detected and four sites where DNA was detected but no salmon were observed. Detectability was < 1 for both methods, and we expected the latter case given that occurrence estimates based on snorkeling were likely to be less precise owing to the effects of water clarity, observer error, and low abundance of juvenile Chinook Salmon in some habitats. The two instances where snorkeling detected juvenile salmon but eDNA did not were surprising. The first site was the West Fork Chena River, which had the largest drainage area among sampled tributaries but the lowest juvenile salmon density (e.g., only six fish were observed at this site via snorkeling). It is possible that with a small amount of DNA and a large volume of water, the density of DNA was low enough that it was not detected by our technique. Moreover, this tributary is near the upstream extent of the range of rearing habitats in the basin; thus, the fish observed here could have been transient. The second site was Rock Creek where eDNA sampling occurred 10 days prior to the snorkel survey owing to poor water clarity and site access conditions (i.e., water level was too high to snorkel). Perhaps juvenile Chinook Salmon are not using all tributary habitats for the entirety of the summer rearing period but are moving in and out of the tributaries with changes in flow conditions. This illustrates a limitation of eDNA in that it is a snapshot of the environment at that moment and may not capture occupancy dynamics over an extended period. Repeated eDNA sampling of tributary habitats throughout the summer rearing period (e.g., Laramie et al. 2015) would better answer this question, but was beyond the scope of the current study.

MANAGEMENT IMPLICATIONS

In conclusion, we found that rapid bioassessment methods to estimate the distribution of a sensitive, juvenile life stage of salmon were useful and will likely become more so under a changing environment. Taken in isolation, the intrinsic potential models, environmental DNA, and snorkel surveys were effective, non-invasive, and low effort methods to assess distributions, but each were limited and a combination of the techniques is the most powerful approach. For example, although the IP model allowed for habitat potential prediction, the eDNA assays (or snorkel surveys) offered an opportunity to validate the model. This is particularly useful to identify locations where fish occur. However, these specific techniques will not always be appropriate given species, ontogeny, and life history (e.g., large fish that move between habitats frequently), but the approach should be easy to modify given the specific situation.

The eDNA approach in particular could provide a useful method for monitoring juvenile salmon use of tributary habitats. Using repeated eDNA sampling with a dynamic occupancy estimation approach would quantify colonization and extinction events between sample periods while accounting for imperfect detection of DNA (Mackenzie et al. 2006). Given advances in next generation sequencing (Guaaratne et al. 2012), and the eDNA assays, a similar occupancy approach could also be used to monitor species assemblages and communities through time (Minamoto et al. 2012; Goldberg et al. 2015). Based on our power analysis and observed detection probability and occupancy estimates, we had high power to detect even small changes in occupancy. However, under conditions such as those we observed, care should be taken in designing monitoring studies as our results indicated more power to detect occupancy increases than decreases. Declines in the proportion of sites occupied are likely to be of more interest for management.

Finally, although each method had drawbacks, the combination of rapid assessment tools provided a great deal of information, both in results and methodological development. It is critical to understand the advantages and limitations of any quantitative technique, especially when those limitations need to inform the interpretation of the results and development of the study design. This project contributes to the field of fisheries by combining the use of three different techniques and understanding how they work together so that they may be applied in other systems with other species and life stages.

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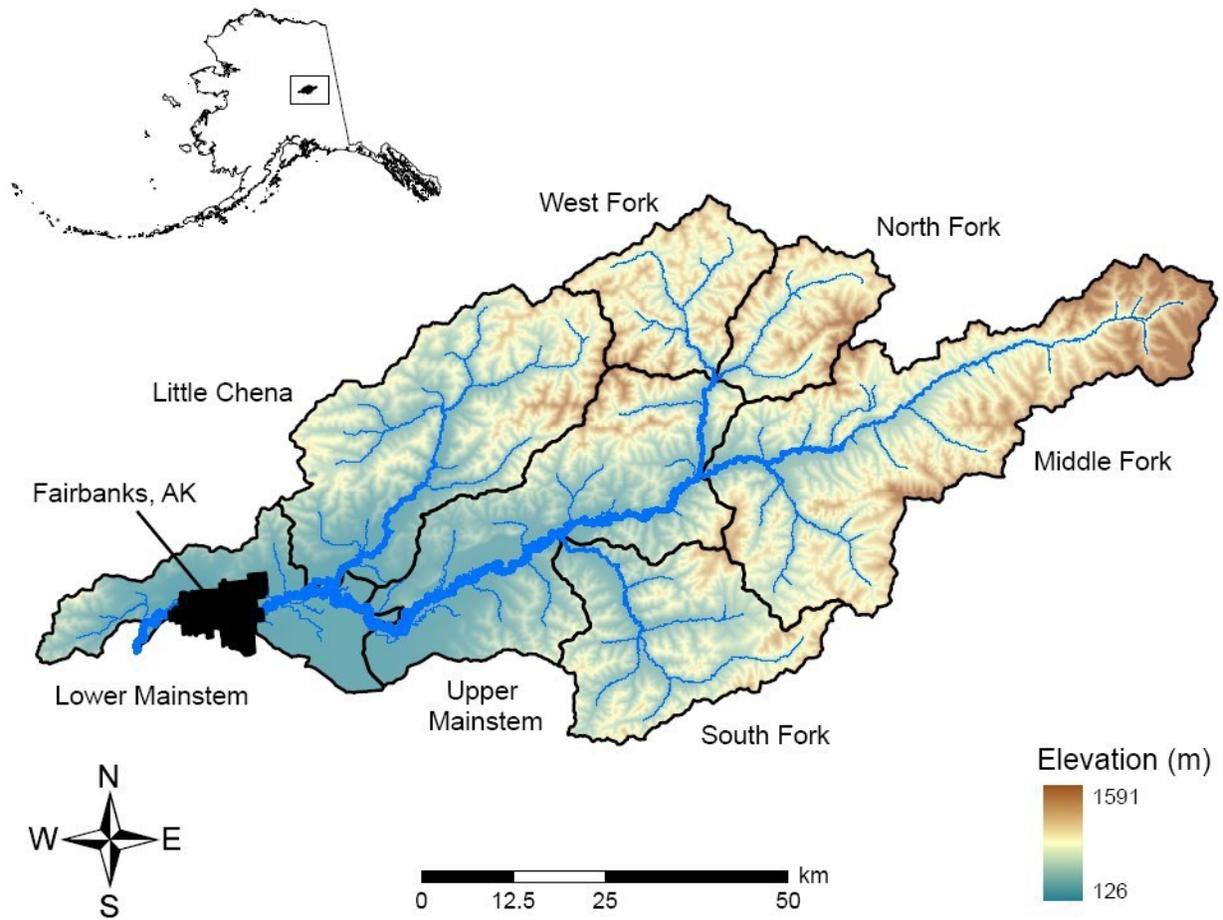


Figure 1.1. Location of the Chena River basin in Alaska (inset). Sub-basins, elevation, and the location of the city of Fairbanks are shown.

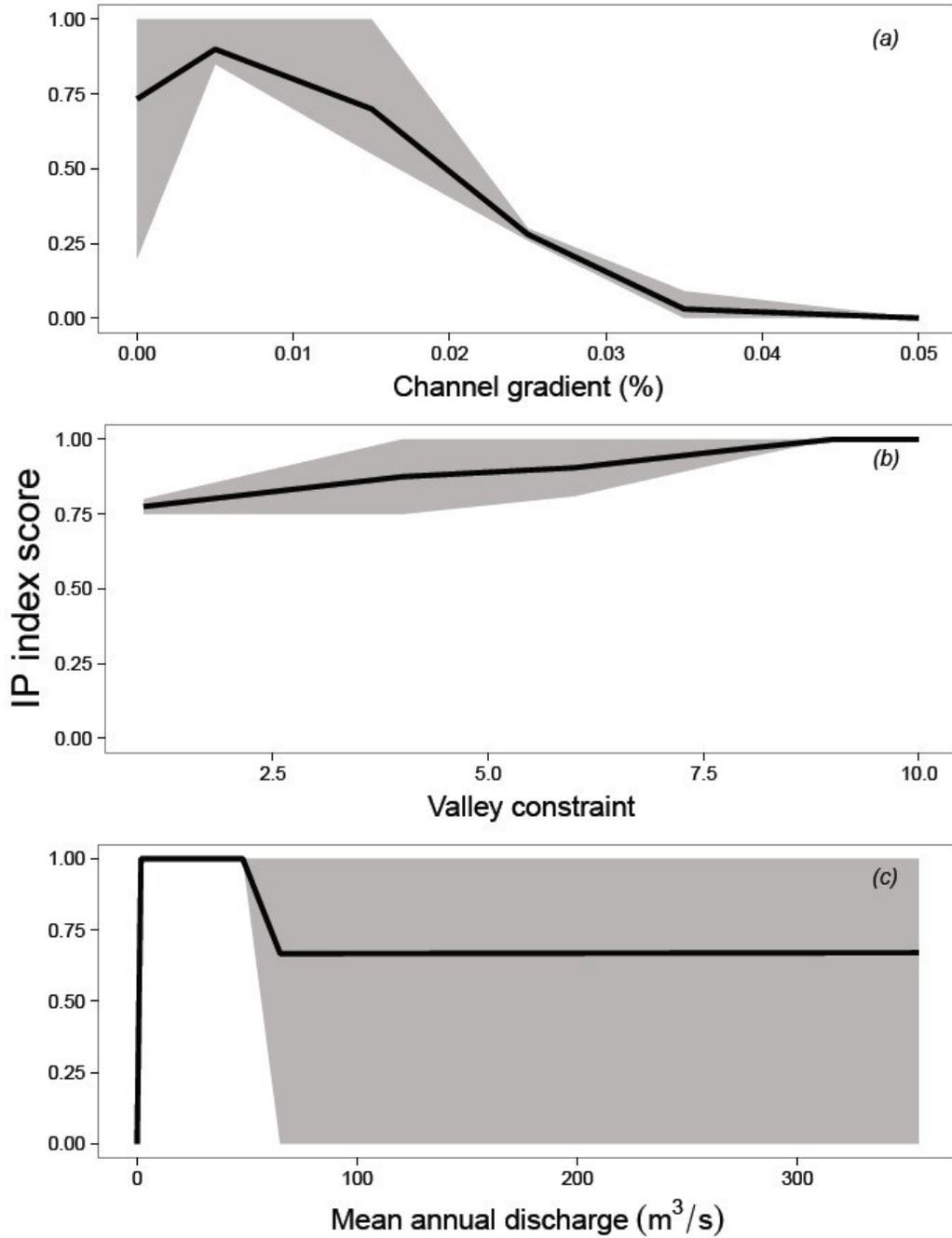


Figure 1.2. Habitat suitability index curves averaged across three previous juvenile Chinook Salmon models (lines) for three geomorphic attributes: (a) channel gradient (%), (b) valley constraint (valley width: bankfull width), and (c) mean annual discharge (m^3/s). Shading represents the range of values predicted from the three models (Sheer et al. 2009, Bidlack et al. 2014).

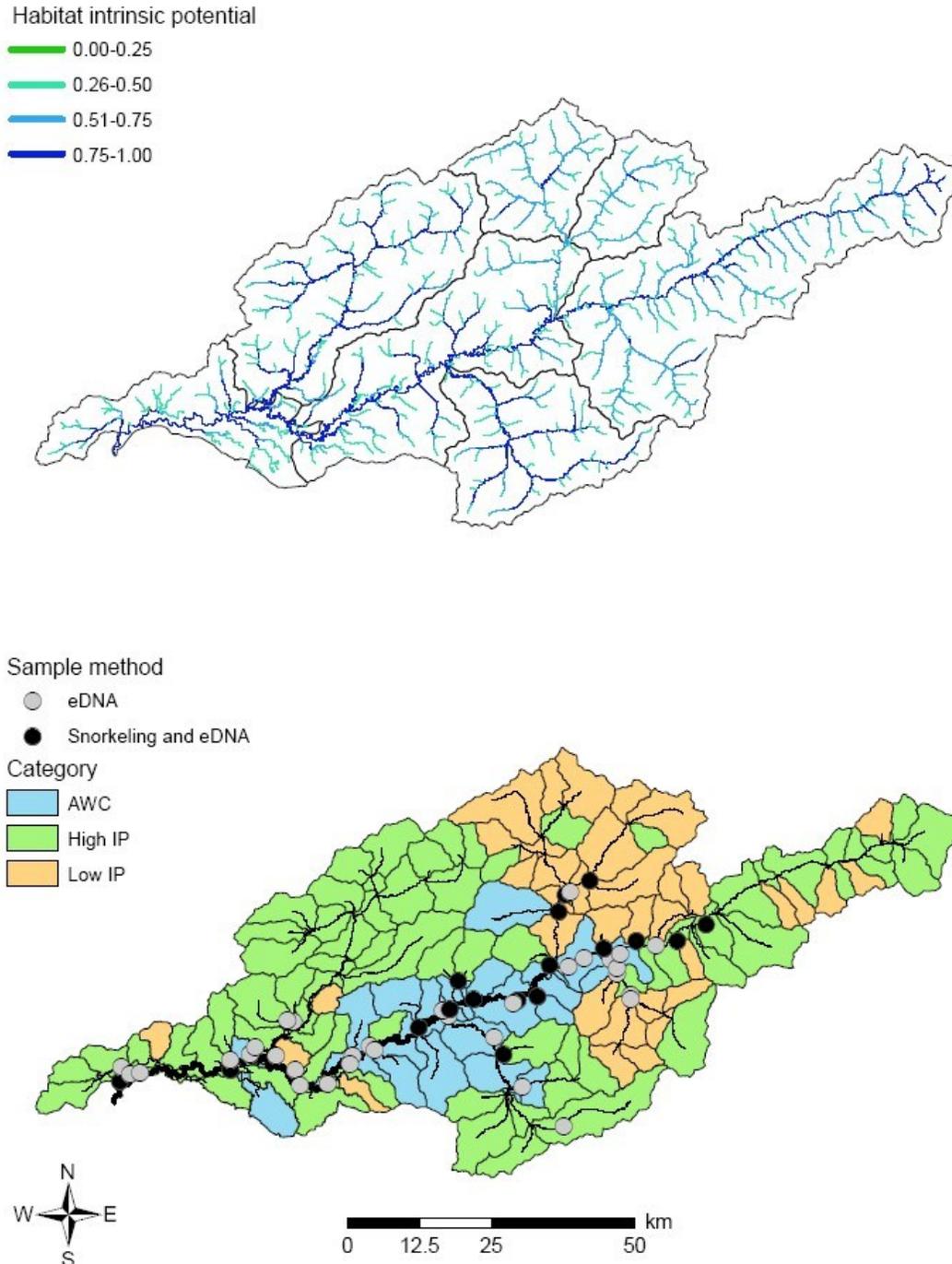


Figure 1.3. Predicted juvenile Chinook Salmon rearing habitat intrinsic potential (IP score) for the Chena River basin, Alaska (upper panel). Lower panel shows tributaries (> 20 km² catchment area) categorized by IP score (high IP \geq 0.75, low IP < 0.75) and known rearing as designated by the State of Alaska Anadromous Waters Catalog (AWC). Open circles indicate sites (i.e., tributaries) sampled using eDNA and closed circles were sites sampled by eDNA and snorkel surveys.

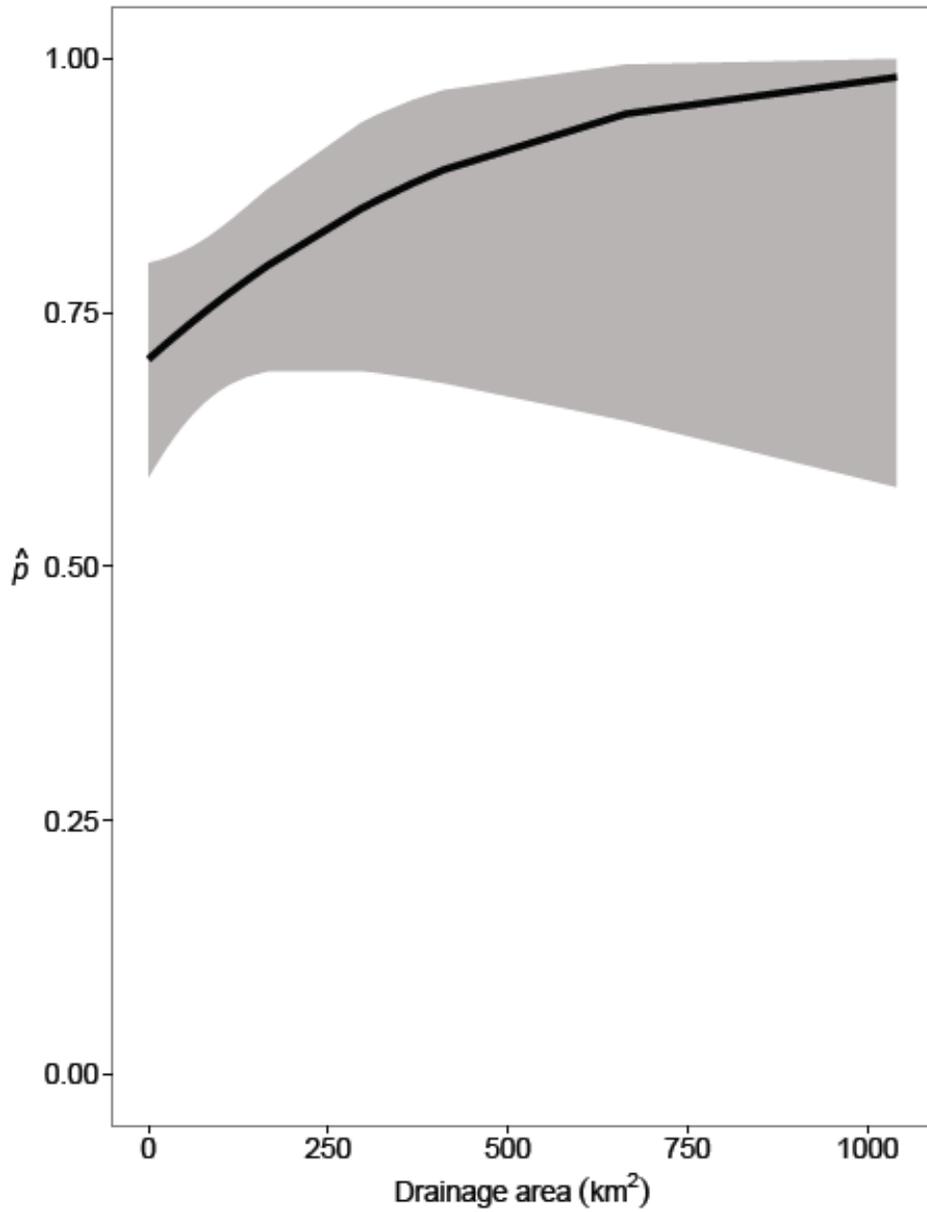


Figure 1.4. Estimated detection probability (\hat{p} ; y-axis) of Chinook Salmon, given that fish are present, based on three replicate eDNA samples as a function of drainage area (km²; x-axis).

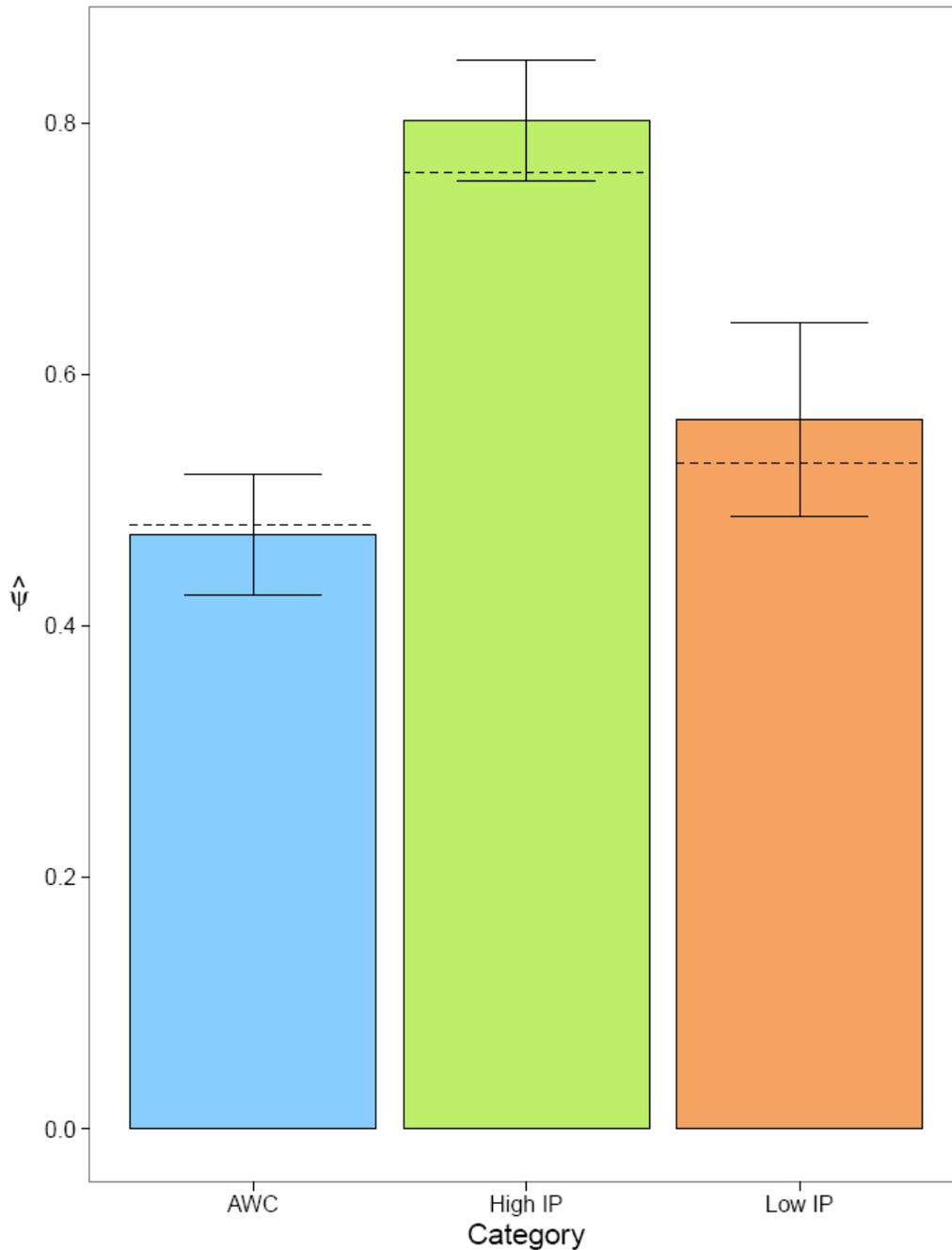


Figure 1.5. Estimates with standard errors from an occupancy model predicting the proportion of sites occupied ($\hat{\Psi}$ -y-axis) as a function of tributary sites categorized by juvenile Chinook Salmon rearing habitat intrinsic potential (high IP ≥ 0.75 , low IP < 0.75) and known rearing as designated by the State of Alaska Anadromous Waters Catalog (AWC). Dashed lines indicate raw estimates (i.e., Ψ) for each category.

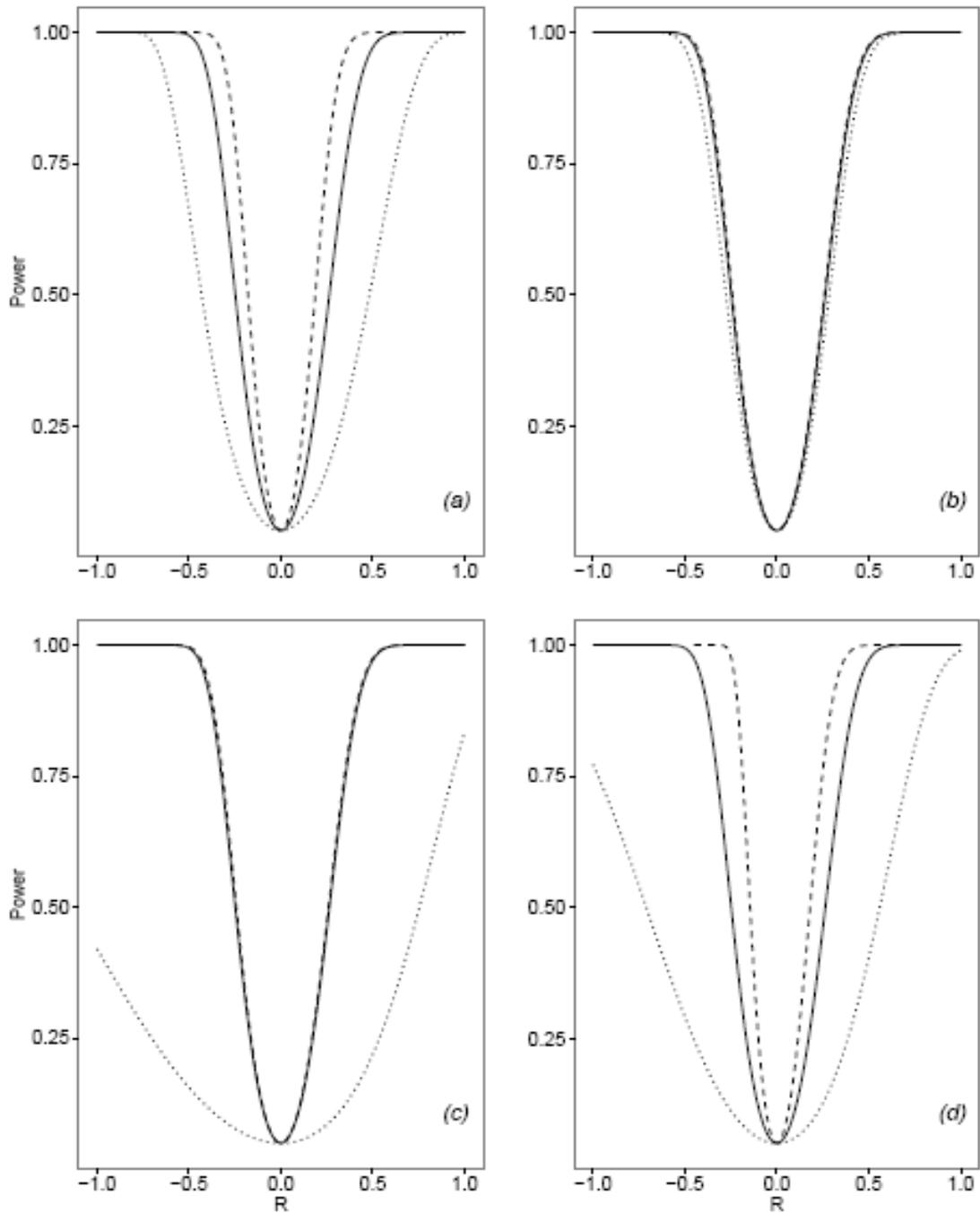


Figure 1.6. Power curves ($\alpha = 0.05$) illustrating the ability to detect proportional changes in occupancy (R) given variation in the number of sample sites (S), number of replicate samples (K), detection probability (p) and proportion of sites occupied ($\hat{\Psi}$). For example, $R = -0.5$ would indicate a 50% decline in site occupancy. The solid line represents a reference case of our data with $\hat{\Psi} = 0.608$ and $p = 0.76$, $K = 3$, $S = 75$. In each panel, one of the parameters changes: (a) $S = 21$ (dot), $S = 150$ (dash); (b) $K = 2$ (dot), $K = 6$ (dash); (c) $p = 0.2$ (dot), $p = 1.0$ (dash); (d) $\hat{\Psi} = 0.2$ (dot), $\hat{\Psi} = 0.8$ (dash).

Table 1.1. Summary of habitat characteristics used to develop a juvenile Chinook Salmon rearing habitat intrinsic potential model (IP) for seven sub-basins within the Chena River basin, Alaska (Figure 1.1). Total stream length (km) categorized as High IP (> 0.75) is shown.

Sub-basin	Drainage area (km ²)	Total stream length (km)	Mean gradient (%)	Mean annual discharge (m ³ /s)	Mean valley constraint	High IP stream km
Little Chena	1026.7	425.8	0.018	14.2	57.4	222.2
Lower Main stem	453.6	285.3	0.016	172.1	278.4	132.4
Main stem	1104.4	467.2	0.013	44.3	83.3	196.1
Middle Fork	1417.3	550.9	0.025	11.7	31.5	138.0
North Fork	330.2	121.3	0.028	5.3	30.4	3.0
South Fork	650.5	260.9	0.016	11.6	35.0	153.0
West Fork	403.5	155.1	0.025	6.0	29.6	12.4

Table 1.2. Results of eDNA surveys and occupancy estimation analysis for juvenile Chinook Salmon in the Chena River basin. The number of sites at which Chinook Salmon were detected or not detected is shown for 2014 and 2015 categorized by IP score (high IP ≥ 0.75 , low IP < 0.75) and known rearing as designated by the State of Alaska Anadromous Waters Catalog (AWC). Raw (Ψ) and estimated ($\hat{\Psi}$) proportion of sites occupied (\pm SE) are shown for each category.

Category	2014		2015			Total		Sites occupied	
	Detected	Not Total detected	Detected	Not Total detected	Detected	Not detected	Ψ	$\hat{\Psi}$	
AWC	5	11	9	4	14	15	0.483	0.473 \pm 0.048	
High IP	10	4	12	3	22	7	0.759	0.80 \pm 0.048	
Low IP	1	4	8	4	9	8	0.529	0.564 \pm 0.077	
Overall	16	19	29	11	45	30	0.600	0.608 \pm 0.03	

Table 1.3. Model selection results for two-stage (detection and occupancy) occupancy models of juvenile Chinook Salmon eDNA in the Chena River basin, Alaska. Models with (§) indicate those with no covariates and (*) indicate a global model with all variables. Parameters in the model are detection probability (p) and occupancy (Ψ). Covariates include drainage area (km^2 ; A), flow (m^3/s ; F), category (AWC, high IP, low IP; C), and sample year (2014, 2015; Y). Models with Akaike information criteria (AIC) weights (w_i) > 0.05 are shown. For each model, K is the number of estimated parameters, and ΔAIC is the difference in AIC relative to the top model.

Stage	Model	K	AIC	ΔAIC	w_i
Detection	$p(\text{A}) \Psi(\cdot)$	3	247.31	0.00	0.41
	$p(\text{F}) \Psi(\cdot)$	3	248.02	0.70	0.29
	$p(\cdot) \Psi(\cdot)\S$	2	249.26	1.95	0.15
	$p(\text{A}+\text{F}) \Psi(\cdot)*$	4	249.3	1.99	0.15
Occupancy	$p(\text{A}) \Psi(\text{C})$	5	245.06	0.00	0.49
	$p(\text{A}) \Psi(\text{C}+\text{F})$	6	247.06	2.0	0.18
	$p(\text{A}) \Psi(\text{A})$	4	248.85	3.8	0.07
	$p(\text{A}) \Psi(\text{C}+\text{Y})$	6	248.86	3.8	0.07
	$p(\cdot) \Psi(\cdot)\S$	2	249.26	4.2	0.06
	$p(\text{A}) \Psi(\text{F})$	4	249.31	4.25	0.06

Table 1.4 Summary of snorkel survey statistics. Sites sampled indicate the number of 50 meter reaches that were sampled within the tributary. The density estimate is equal to the abundance divided by the total stream length that was sampled (upstream extent).

Name	Sub-basin	Lat	Long	Category	Reaches sampled	Abundance	Upstream extent (m)	Density (fish/m)
Angel Creek	Mainstem	-146.21	65.02	Low IP	3	-	350	-
Chena Slough	Lower Mainstem	-147.49	64.84	AWC	3	-	350	-
Cripple Creek	Lower Mainstem	-147.92	64.84	High IP	3	-	350	-
Flat Creek	Mainstem	-146.77	64.86	AWC	9	66	1250	0.053
Fourmile Creek	Mainstem	-146.57	64.89	AWC	3	-	350	-
Horner Creek	Mainstem	-146.95	64.84	AWC	-	-	-	-
Lower Colorado Creek	Mainstem	-146.65	64.89	AWC	6	75	800	0.094
Lower Munson Creek	Middle Fork	-146.08	64.95	AWC	-	-	-	-
Marten Creek	South Fork	-146.49	64.81	High IP	-	-	-	-
Mastadon Creek	Mainstem	-146.57	64.89	AWC	4	16	500	0.034
Monument Creek	North Fork	-146.08	65.06	Low IP	12	172	1700	0.101
Rock Creek	Mainstem	-146.34	64.90	AWC	5	558	650	0.859
Steele Creek	Lower Mainstem	-147.48	64.843	High IP	-	-	-	-
Stiles Creek	Mainstem	-146.28	64.93	High IP	4	6	500	0.012
West Fork Chena River	West Fork	-146.18	65.04	Low IP	5	6	650	0.009
Demar Creek	Middle Fork	-145.94	64.95	Low IP	-	-	-	-
Lohi Creek	Middle Fork	-145.73	64.97	High IP	-	-	-	-
Ottertail Creek	Middle Fork	-145.87	64.95	Low IP	-	-	-	-
Smallwood Creek	Little Chena	-147.24	64.89	High IP	-	-	-	-
Unnamed Creek B	Middle Fork	-146.07	64.95	Low IP	2	-	200	-
1503	Middle Fork	-146.01	64.94	AWC	3	-	350	-

Table 1.5. Confusion matrix comparing the number of sites at which juvenile Chinook Salmon in the Chena River basin, Alaska were detected or not detected based on eDNA and snorkel sampling.

Snorkel surveys	eDNA	
	Detected	Not detected
Detected	4	2
Not detected	4	5

Conclusions

This project developed and evaluated the utility of a rapid assessment method that integrates habitat intrinsic potential (IP) modeling, environmental DNA (eDNA) occupancy estimation, and snorkel surveys to better delineate distributions of juvenile Chinook Salmon in the Chena River, Alaska. For each method, I considered the method's ease of use and effectiveness, as well as its ability to address challenges (e.g., cost, logistics, species detection) associated with identifying species distributions. The main findings of this work were as follows:

- The IP model predicted that 41% (931 km) of the Chena River basin had high potential as juvenile Chinook Salmon rearing habitat ($IP \geq 0.75$), 14.5% of which was along the main stem with the remainder in tributary habitats.
- At the tributary scale, 58% of all catchments were categorized as high IP ($IP \geq 0.75$), 21% as low IP ($IP < 0.75$), and 21% as known to be used by juvenile Chinook Salmon based on the State of Alaska Anadromous Waters Catalog (AWC).
- Of tributaries sampled for eDNA, 61% (± 3.0) were estimated to be occupied by juvenile Chinook Salmon. For previously unsampled sites, 80.0% (± 9.6) of high IP, 56.0% (± 15.0) of low IP, and 47% (± 10.0) of AWC sites were estimated to be occupied.
- Detectability of Chinook Salmon DNA in the Chena River using eDNA occupancy estimation methods was high (0.76 ± 0.02) and increased with drainage area.

- I found a greater power to detect increased proportional changes in occupancy (R) when I varied the number of sample sites (S), the number of replicate samples (K), the detection probability (p), and occupancy (ψ), relative to decreased changes.
- Water clarity and high flows were major limiting factors for snorkel surveys.
- The upstream extent of juvenile salmon use of tributaries ranged from 200 to 1,350 m from tributary confluences.

The GIS-based intrinsic potential model (IP; Burnett et al. 2007) was straightforward to develop once I became more comfortable with ArcGIS (ESRI 2011) and the Netmap tools extension (Benda et al. 2007). The Chena River NetMap model had been previously developed for another project (Huntsman et al. *UAF unpublished data*). I parameterized three suitability curves for juvenile Chinook Salmon based on literature values (Sheer et al. 2009; Bidlack et al. 2014) but found considerable uncertainty among the models. To account for this uncertainty, I averaged the three models prior to application to the Chena River basin but took note that once the model was applied, model accuracy cannot be determined unless there is on the ground sampling to confirm model predictions. I found that my averaged model did estimate juvenile Chinook Salmon habitat. Although an averaged model may be a useful first pass approximation, the relationship between averaged IP scores and juvenile tributary habitat use might not be as strong in other basins owing to habitat and flow variability. I recommend that IP models should be refined for specific regions, which may include incorporating alternate attributes (e.g., glacial input, large wood) that are important in a specific basin.

For future IP work in the Chena River, I propose a comparison of the distribution of adult spawning habitat quality (e.g., Huntsman et al. *UAF unpublished data*) with juvenile

habitat in an effort to evaluate the degree of spatial overlap. Proximity to the spawning grounds might be a useful attribute for future rearing habitat models. Outside of the Chena River, I suggest mapping other important interior basins such as the nearby Salcha, Chatanika, and Goodpaster rivers. For example, one could apply the Chena River IP model to the Salcha River because they are quite similar in physical characteristics. Overall, a map of juvenile Chinook Salmon habitat potential across the state of Alaska would be useful, with some on-the-ground validation, for protecting this precious resource and would complement the AWC by providing not just presence but a metric of habitat quality.

I used eDNA assays to assess which tributary habitats were occupied by juvenile Chinook Salmon (Jerde et al. 2011). I found that the effort required to collect the samples in the field was quite low, but effort required to process the samples was much higher and depended on the method of extraction and presence of inhibitors in a sample (Renshaw et al. 2014). The primers are a key component for Chinook Salmon eDNA detection assay and had fortunately been previously developed (Laramie et al. 2015). Because of the low collection effort, multiple sites could be sampled per day to achieve coverage of a large area. No prior information is needed about a site before you can take a water sample and analyze it, provided species-specific primers are available (Wilcox et al. 2013).

For further studies using eDNA, I suggest surveying additional unsampled tributaries in the Chena River, with particular focus on the Little Chena River sub-basin. The IP model predicted a relatively high proportion of stream reaches in this sub-basin to be high IP. Owing to high turbidity likely resulting from land use, the Little Chena River basin is considered poor Chinook Salmon spawning and rearing habitat. However, I found Chinook Salmon DNA to be present at all three tributaries sampled in the Little Chena River suggesting that salmon indeed

are using these habitats. More information on the relationship between IP score, juvenile salmon presence, and land use practices is warranted.

Because the eDNA method detects DNA from locations upstream of the sample point, repeated sampling at systematic intervals moving up the tributary would be a useful alternative to snorkeling or other active techniques to delimit the distribution of habitat use. Moreover, I think it would be interesting to select a few tributaries to sample repeatedly throughout the summer to quantify the point at which juveniles start using the tributaries and attempt to detect movement in and out of tributaries. Additionally, it would be advantageous to collect water samples throughout the basin and use next generation sequencing to quantify aquatic species composition in Chena River tributaries.

Snorkeling required much more effort than initially anticipated, both in energy expended and survey time. Sampling over large extents (i.e., many km up a tributary) is likely not feasible using these methods. Another major challenge we faced was water clarity. Due to physical habitat characteristics, visibility was low in many tributaries, making them impossible to snorkel and observe fish. However, in clear-water, I was able to identify and enumerate juvenile Chinook Salmon without causing stress to the fish, confirming the utility of this observational approach given suitable conditions. In most tributaries, snorkel surveys were not an appropriate technique owing to low water clarity. I would suggest using an alternative method of capture such as baited minnow traps or electrofishing where snorkeling cannot be conducted. To quantify the upstream extent of juvenile salmon tributary use, eDNA could be employed systematically to sample up the tributary until the analysis shows no detectable DNA. The practitioner could then return to the upstream extent and intensively sample downstream using snorkeling (if the water is clear) or an alternative capture method. This method might allow for

fine tuning of extent estimates and would fulfill the requirements to nominate the water body for the Anadromous Waters Catalog.

Overall, I found that rapid assessment methods to estimate the distribution of juvenile Chinook Salmon were useful, and suggest that such techniques will become more valuable in the future under anthropogenic land use and climate change. These rapid assessment techniques can be implemented much easier than traditional techniques because of the reduced need for personnel and equipment. Such techniques will be especially useful in Alaska owing to its extreme remote nature and because climate change is occurring rapidly in this region (Hinzman et al. 2005). To assess distributions independently, the intrinsic potential model, environmental DNA, and snorkel surveys were effective, non-invasive, and easily implemented. However, each method has its advantages and limitations, and a combination of the techniques is likely to be the most powerful approach. I found that the combination of techniques provided critical information both in results and methodological development. This research contributes to the field of fisheries by understanding these techniques and how they work together so that they may be applied in other systems with other species and life stages.

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Appendices

Appendix 1A. 2014 IACUC approval



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Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

July 24, 2014

To: Jeffrey Falke
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [619748-2] Distribution of juvenile Chinook salmon in the Chena River basin, Alaska

The IACUC reviewed and approved the Revision referenced above by Designated Member Review.

Received:	July 11, 2014
Approval Date:	July 24, 2014
Initial Approval Date:	July 24, 2014
Expiration Date:	July 24, 2015

This action is included on the August 14, 2014 IACUC Agenda.

All documents and personnel are approved with the exception of Huntsman who must complete CITI IACUC and Fish module training before participating in field work.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*

Appendix 1B. 2015 IACUC approval



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Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

April 2, 2015

To: Jeffrey Falke
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [619748-3] Distribution of juvenile Chinook salmon in the Chena River basin, Alaska

The IACUC has reviewed the Progress Report by Designated Member Review and the Protocol has been approved for an additional year.

Received:	March 30, 2015
Initial Approval Date:	July 24, 2014
Effective Date:	April 2, 2015
Expiration Date:	July 24, 2016

This action is included on the April 9, 2015 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures detailed in the form 005 "Reporting Concerns".*