COMPARISON OF ENVIRONMENTAL DNA AND UNDERWATER VISUAL

COUNT SURVEYS FOR DETECTING JUVENILE COHO SALMON

(*ONCORHYNCHUS KISUTCH*) IN RIVERS

By

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A Thesis Presented to

The Faculty of California State Polytechnic University, Humboldt

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Natural Resources: Fisheries

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December 2022

ABSTRACT

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Environmental DNA (eDNA) has developed into a useful tool for determining the distribution of rare aquatic species, but relatively few studies have directly compared the detection probabilities of this method with other conventional survey techniques. These comparisons can inform which method may be better suited to address study objectives. In this study, the overall goal was to compare the ability of eDNA and underwater visual count (UVC) surveys to detect juvenile coho salmon (*Oncorhynchus kisutch*), a species of conservation concern at the southern extent of its geographic range. Specifically, I address two objectives: (1) compare the ability of eDNA and UVC surveys to detect coho salmon and the influence of environmental covariates on detectability and (2) evaluate the utility of eDNA concentrations and habitat covariates to predict the count of coho salmon within small pools. Water samples for eDNA analysis and snorkel surveys were conducted at 96 pools across 25 stream reaches in the Smith River basin, California. I used multi-scale occupancy models to estimate method-specific detection probabilities (p) and the effect of habitat covariates, including basin area (as a proxy for discharge), residual pool depth, and large woody debris. Results showed that eDNA and UVC surveys had a high degree of agreement in detecting the presence of coho salmon at both

the pool scale (93% agreement) and reach scale (80% agreement), however there were several occasions where only one method detected coho salmon. The top occupancy model, identified using Akaike's information criterion, indicated that the detection probabilities were best predicted by method, basin area, residual pool depth, and an interaction between method and basin area. Under median habitat conditions, detection probabilities were similar and high for both methods (p_{eDNA}=91%, p_{UVC}=89%). Residual pool depth had a slight positive effect on p_{eDNA} and p_{UVC}. Detection probabilities for both methods were affected negatively by increasing basin size, but p declined more substantially for eDNA; at the highest basin areas, p_{eDNA} =40% compared to p_{UVC}=78%. Finally, eDNA concentrations were a poor predictor of coho salmon count in small pools. The absence of a relationship between eDNA concentrations and fish counts is contrary to other studies and may have resulted as a consequence of the relatively small differences in counts observed between pools, which ranged from 0 to 210 individuals. Overall, this study illustrates that eDNA methods were as sensitive as UVC surveys for detecting coho presence under most conditions but could not be used to produce reliable estimates of the average observed count of the target species in this system. Therefore, these findings support the use of eDNA methods for monitoring the distributions of a rare species but indicate that implementation should be guided by study objectives and local environmental conditions.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

Freshwater biodiversity is declining world-wide and extensive, ongoing conservation and restoration efforts are underway to help stop this decline (Dudgeon et al. 2006; Reid et al. 2019). Conservation projects typically rely on monitoring programs for assessing population trends and collecting relevant ecological data to make appropriate management decisions or to evaluate the effects of past decisions (Nichols and Williams 2006; Lovett et al. 2007). One of the key parameters for monitoring Pacific salmonids is tracking geographic distribution through space and time (McElhany et al. 2000). Monitoring this spatial structure is challenging when species are hard to observe, have broad spatial distributions, occur at low abundance, or inhabit remote areas (Albanese et al. 2011; MacKenzie et al. 2018). These factors increase the chances of failing to detect a species that is present, and such imperfect detection can negatively bias estimates of species distributions. To address these challenges, monitoring programs often require extensive survey efforts and may utilize multiple survey methods to maximize detectability for more accurate quantification of population spatial structure (Nichols et al. 2008).

Underwater visual count (UVC) surveys are commonly used to monitor the distribution and abundance of aquatic species (Hankin and Reeves 1988; Thurow 1994). Underwater visual surveys via direct (e.g., snorkeling) or indirect (e.g., camera stations) observation are often used in remote areas due to minimal gear requirements or when

environmental conditions (e.g., deep water or high conductivity) limit the effectiveness of other methods such as seining or electrofishing (Thurow 1994; Albanese et al. 2011). The minimally invasive nature of UVC surveys makes them well adapted for sampling of sensitive or imperiled species such as those listed under the United States Endangered Species Act. However, UVC surveys are prone to imperfect detection especially when abundance is low, species are morphologically similar, or when field observations are limited by water clarity, depth, or habitat complexity (Gu and Swihart 2004; Thurow et al. 2012; MacKenzie et al. 2018).

Environmental DNA is developing as a method for assessing the distribution and relative abundance of aquatic species (Thomsen and Willerslev 2015). Environmental DNA (eDNA) is DNA from an environmental sample (e.g., water, air, soil) that has been shed from an organism (e.g., mucous, scales, epithelial cells, etc.) which can then be collected from the environment and analyzed for species-specific DNA without directly interacting with the target organism. Several studies suggest that eDNA methods are more rapid, cost effective, and sensitive than conventional survey methods, particularly when surveying for rare or endangered species (Laramie et al. 2015; Strickland and Roberts 2019; Sutter and Kinziger 2019; Spence et al. 2021; Yu et al. 2021). The benefits of using eDNA methods for ecological monitoring are still being explored as the method was only first applied to aquatic species in 2008 (Ficetola et al. 2008). Since then, the cumulative number of publications using eDNA methods has increased rapidly each year (Rodríguez-Ezpeleta et al. 2021).

Several factors have been shown to impact species detection with eDNA methods such that eDNA monitoring strategies should be tailored to the area and species of interest (Spence et al. 2021). The relative quantity and distribution of eDNA in a system is strongly dependent on the behavior, abundance, and DNA shedding rate of the target organism (Jane et al. 2015; Baldigo et al. 2017; Andruszkiewicz Allan et al. 2021; Wood et al. 2021). Additionally, dispersal of eDNA in rivers is primarily driven by the hydrological characteristics (e.g., discharge, velocity, substrate composition) of the study area which can lead to considerable variability in the transport, settling, and decay dynamics between and within drainages (Barnes et al. 2014; Jane et al. 2015; Barnes and Turner 2016; Shogren et al. 2017).

Occupancy modeling frameworks have been increasingly applied to eDNA datasets to account for the imperfect detection of DNA in water samples and the influence of environmental factors on occupancy and detectability (e.g., Schmelzle and Kinziger 2016; Sutter and Kinziger 2019; Smith and Goldberg 2020). The hierarchical nature of eDNA surveys fits easily into a multi-scale occupancy framework to estimate occupancy patterns at multiple spatial scales while accounting for environmental and methodological covariates (Nichols et al. 2008; MacKenzie et al. 2018). For example, multi-scale occupancy models can be used to estimate and compare the detection probabilities of different survey methods, providing critical information for survey design and method-specific effectiveness (Nichols et al. 2008).

Study designs that incorporate eDNA and other survey methods are relatively common, but the formal quantification and comparison of method-specific detection probabilities is relatively limited, particularly within freshwater river systems (e.g., Castañeda et al. 2020; Spence et al. 2021). Fediajevaite et al. (2021) found that of 535 papers identified as using eDNA methods, 194 described comparisons of eDNA to conventional survey methods. Of those, however, only 18 (9%) provided a quantitative comparison of eDNA and conventional survey methods via estimation of method-specific detection probabilities. Additionally, despite the clear importance of implementation cost to management agencies, only 19 of the 194 papers used the relative costs of survey methods as a basis for comparison (Fediajevaite et al. 2021). Given the rarity of robust comparative studies, additional comparisons of UVC and eDNA in freshwater river systems are needed to inform management decisions and to better establish the efficacy of eDNA as a potential tool for monitoring.

The goal of this study was to compare eDNA and UVC surveys for monitoring the spatial distribution of naturally spawned juvenile coho salmon (*Oncorhynchus kisutch*) in the Smith River basin, California. The population of coho salmon inhabiting the Smith River are considered part of the Southern Oregon/Northern California Coast Evolutionarily Significant Unit and are currently listed as threatened under the Endangered Species Conservation Act of the United States of America (*Endangered Species Conservation Act* 1973). The California Department of Fish and Wildlife (CDFW) uses UVC to determine the spatial distribution of juvenile coho salmon in the

Smith River each summer (Walkley and Garwood 2017). I integrated eDNA collections into CDFW's pre-existing survey protocols over two survey seasons (2020-2021) for a robust comparison of eDNA and UVC methods. This study was designed to address two objectives: (1) to compare the ability eDNA and UVC surveys to detect coho salmon and (2) to evaluate the potential for using eDNA concentrations and habitat covariates to predict the count of coho salmon within small pools. These objectives were used to evaluate the tradeoffs of utilizing eDNA methods for determining distribution and relative abundance patterns of a rare species across a broad spatial scale when compared to a well-established survey method.

METHODS

All field protocols for this study were approved by Cal Poly Humboldt's Institutional Animal Care and Use Committee (IACUC, No. 2020F57E).

Study site

The Smith River is located in Northern California and Southern Oregon, and is composed of three major branches (North, Middle, and South forks) and two sub-branches (Mill and Rowdy creeks; Figure 1). The Smith River basin encompasses 1,862 km^2 ranging from sea level to 1,954 m. Nearly all of the basin (98%) is within the Klamath-Siskiyou mountain ranges and has a rugged, complex topography with only 2% of the basin within the coastal plain (Walkley and Garwood 2017). The Smith River is the largest free-flowing coastal river in California providing unrestricted access for anadromous and resident salmonids (Garwood and Larson 2014).
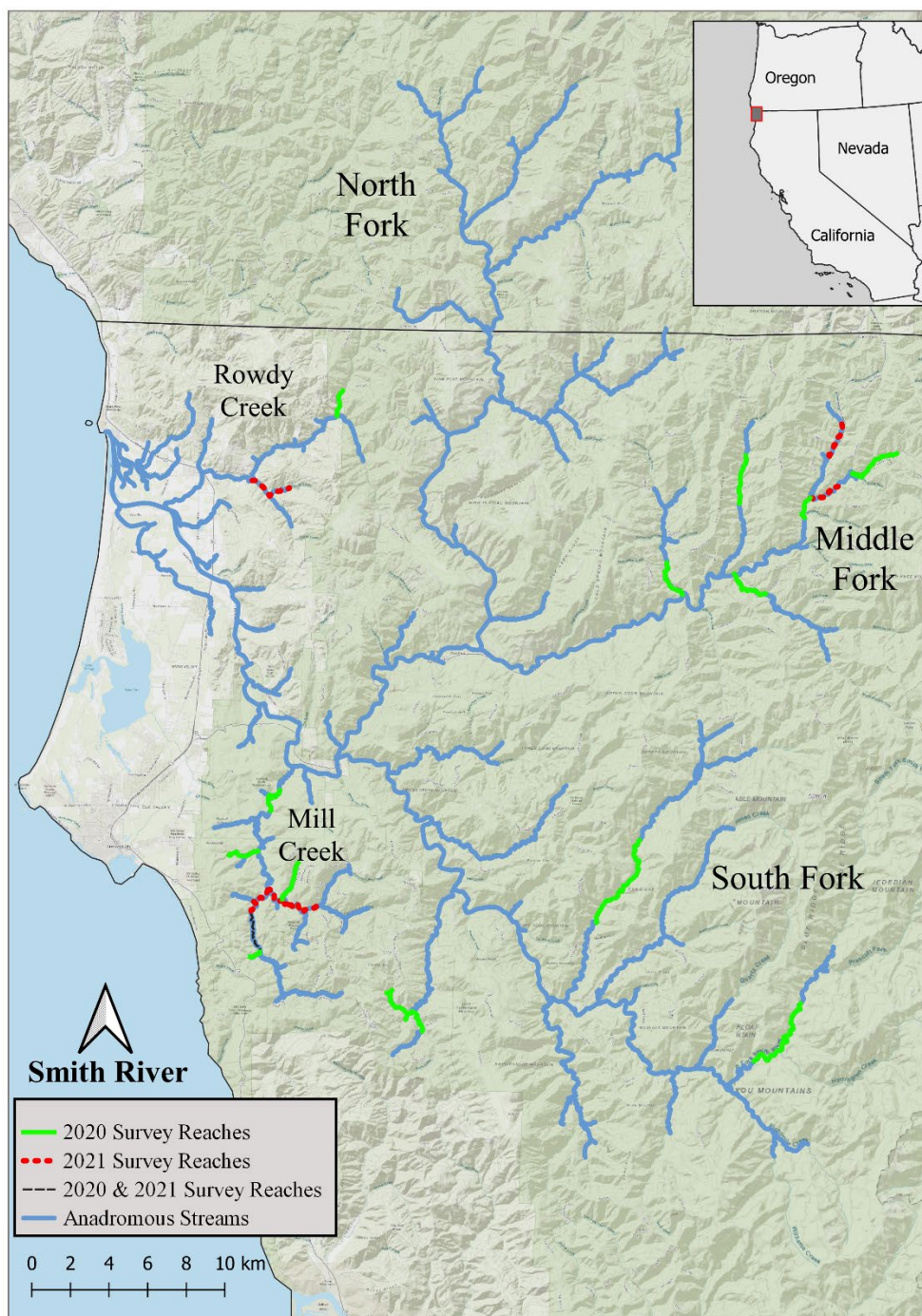
Figure 1. The anadromous rearing habitat (blue lines) of the Smith River basin (California, USA) and the location of the stream reaches that were sampled in 2020 (green lines; 18 reaches), 2021 (red dotted lines; 6 reaches), or in both years (black dashed line; 1 reach) to compare the ability of eDNA and UVC surveys to detect coho salmon.

Field methods

Snorkel (hereafter UVC) surveys of the Smith River basin were conducted in July and August of 2020 and 2021 as part of the CDFW Coastal Salmonid Monitoring Program (Garwood and Ricker 2016; Walkley and Garwood 2017). The total amount of juvenile salmonid rearing habitat in the Smith River basin was divided into 166 sampling units (hereafter reaches) that are approximately one to three km in length (Figure 1; Garwood and Larson 2014). Survey reaches for each year were selected using a Generalized Random Tessellation Stratified (GRTS) sampling design. Over the 2020 field season, travel and overall survey effort were restricted due to COVID-19 such that no reaches were drawn in the North Fork and sampling intensity was lower than previous years (23 reaches compared to a typical average of 65). To augment the 2020 sample collection, a subset of seven additional reaches were surveyed for eDNA from the broader 2021 survey, but these reaches were selected non-randomly by prioritizing reaches with known high abundances of coho salmon to ensure a broader range of observed fish counts. A total of 29 unique reaches (23 in 2020 and 7 in 2021, but one reach surveyed in both years) were surveyed using UVC and eDNA methods. Four reaches were on the main stem of the Smith River where survey methods differed from those in all other survey reaches. Because of the differences in survey methods, I excluded all mainstem reaches from the analysis, leaving 25 survey reaches for the comparison of UVC to eDNA.

Systematic sampling was used to select survey pools within a survey reach. Teams of two to four divers surveyed every other pool that met the minimum habitat requirements, as described by Garwood and Ricker (2016). A coin flip decided which of the first two pools was the start of the survey. Pools were surveyed by conducting two independent census counts and then every other upstream pool was surveyed systematically with the next three surveyed pools only getting a single pass (hereafter referred to as intermediate pools); this sequence (i.e., 2-0-1-0-1-0-1-0) was repeated for the remainder of the reach (Figure 2). For each double pass pool, two divers independently and sequentially surveyed the pool, allowing approximately five minutes between dives. When surveying a pool, divers proceeded upstream, examined the entire width of the pool, and recorded the number of juvenile coho salmon present. Coho salmon were identified by their distinct sickle-shaped anal fin that has a black and white leading edge. Divers also recorded the number of large woody debris (LWD; >30 cm in diameter), the residual pool depth (RD; the pool depth at extreme base flow conditions), the total pool length, and a representative measure of the average pool width. On average, individual survey pools were 157 m apart and the double-pass pools were 536 m apart. Additionally, the contributing basin area (BA) to each survey reach was used as a proxy for river discharge as the two measures are assumed to scale geometrically (Galster 2007). The BA values were obtained using the StreamStats application (U.S Geological Survey 2016) and assumed to be constant for each survey reach .
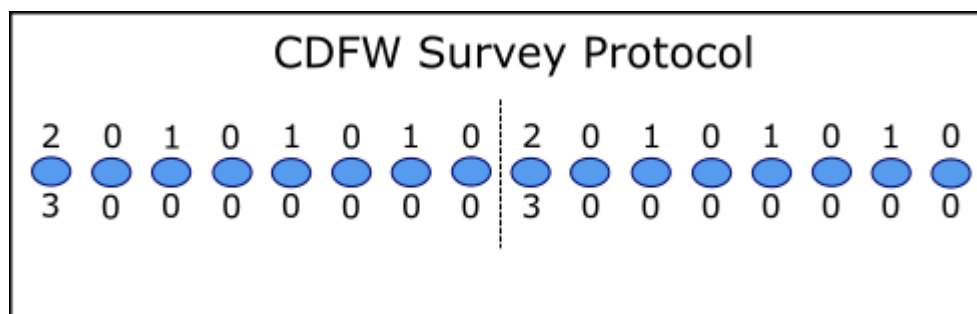
Figure 2. Graphic representation of the CDFW UVC survey protocol and eDNA collection procedures for sequential pools within a sampled stream reach (blue circles). The standard dive procedure (top) has a repeating pattern of double pass (2), single pass (1), and skipped pools (0) that continues until the end of the survey reach. I incorporated triplicate eDNA collections (3) at every double pass pool (bottom).

Water samples for eDNA analysis were collected at every double-pass survey pool to compare the two survey methods within the same pools (Figure 2). Due to limited supplies, there were some occasions where the eDNA collection design was altered to allow for full spatial coverage of a reach. For example, eDNA collections occurred at every other double dive survey pool when reach lengths were greater than two kilometers or when many pools were expected (e.g., $n_{pools} > \sim 30$). To minimize the potential for contamination, all water samples were collected prior to divers entering a pool.

At each pool, three 1-liter water samples were collected using single-use Whirl-Pak bags (Nasco) at the downstream end of the pool. Water grabs were taken by drawing the bag along the surface, and water was filtered immediately in the field across 0.45-micron cellulose nitrate filters (Cytvia; catalog number: 10401170) held in filter funnels (Thermo Scientific™ Nalgene™ Single-Use Analytical Filter Funnels, catalog number: 09-740-30K). Filter funnels were held in a filtration manifold which allowed up to four samples to be filtered simultaneously using a manual vacuum pump. Filter support pads

(MilliporeSigma™ catalog number: AP1003700) were used to ensure equal filtration across the surface of the filter. A field blank was collected at least once per survey day by filtering 1-liter of store-bought drinking water. Field blanks were processed the same as the other samples and served as comprehensive contamination controls. After filtration, filters were folded with sterilized forceps and placed into 2 ml microcentrifuge tubes (Eppendorf catalog number: 022431048) containing 360 µL of cell lysis buffer (QIAGEN buffer ATL, catalog number: 939011). Samples remained unfrozen for a maximum of three days post-filtration due to the remote nature of some survey locations but were stored at -20°C upon returning from the field. To prevent contamination, forceps were sterilized in a 10% bleach solution and new disposable gloves were worn when placing filters into storage vials. Upon returning from the field, filter cups and bases were sterilized in a 10% bleach solution before being rinsed with fresh water and fitted with a new filter pad and filter for re-use.

Molecular methods

All DNA extractions were conducted in a dedicated laboratory that was maintained to ensure that only low concentrations of DNA were present. All work surfaces and extraction tools (i.e., benches, centrifuges, and racks) were sterilized with UV light and researchers could not enter if they had been exposed to any high concentrations of DNA (e.g., from running PCR reactions). The DNA was extracted directly from filters using the QIAGEN DNeasy Blood and Tissue Kits (69504) following the manufacturer's instructions with three exceptions: 1) I used 360 µl of buffer ATL for

sample preservation and 40 µl proteinase K (Schmelzle and Kinziger 2016), 2) QIAGEN's QIAshredders were used to ensure lysate homogenization, and 3) during the final elution step, 100 µl of elution buffer was used to increase the final DNA concentration of the elution. All extractions were completed within three months of field collection and extracted DNA was stored at -20 °C.

The concentration of eDNA in a sample was determined using digital droplet PCR (ddPCR) with the Bio-Rad QX200 Droplet Digital PCR System (catalog number: 1864001). Each ddPCR reaction was run in duplex for (1) coho salmon (*Oncorhynchus kisutch*) using a lock-nucleic acid assay that targets a 114 base pair sequence of the mitochondrial cytochrome b region, and (2) Chinook salmon (*Oncorhynchus tshawytscha*) using an assay that targets a 131 base pair sequence of the mitochondrial cytochrome b region. The coho salmon assay included an Integrated DNA Technologies PrimeTime qPCR Probe with 5' HEX reporter dyes and quenchers of ZEN / Iowa Black FQ from Spence et al. (2021), which is a modified version of the original design found in Pilliod and Laramie (2016). The Chinook salmon assay was a TaqMan minor groove binding probe labeled with FAM and a nonfluorescent quencher developed by the U.S. Forest Service National Genomics Center for Wildlife and Fish Conservation at the Rocky Mountain Research Station, Missoula, Montana.

Both coho and Chinook salmon assays were tested for specificity to their respective species and against several closely related non-target species that occurred in the study area. Tests of assay specificity found no evidence that either assay amplified

non-target DNA from two potentially co-occurring species (steelhead [*Oncorhynchus mykiss*], coastal cutthroat trout [*Oncorhynchus clarkii clarkii*]). However, there was notable background fluorescence in the Chinook salmon channel when coho salmon DNA concentrations were high, but there was no effect of the Chinook salmon assay on the coho salmon channel. Based on the limited reliability of the Chinook assay, the Chinook data were excluded from further consideration.

Each ddPCR reaction mix was comprised of 900 nM forward primer, 900 nM of reverse primer, 250 nM probe, 5 µl of ddPCR Multiplex Supermix (Bio-Rad catalog number: 12005911), 0.2 µl of 300 mM dithiothreitol (Bio-Rad catalog number: 12012171), 15 µl of DNA template to maximize the probability of target DNA presence in the analyte (Rees et al. 2014; Doi et al. 2015a), and water to bring the total volume to 22 µl. Each reaction mix contained equal amounts of primers and probes for both coho salmon and Chinook salmon. Then, for each sample, 20 µl of the total reaction mix and 70 µl of Bio-Rad droplet generator oil (Bio-Rad catalog number: 1864006) were placed into individual wells of a Bio-Rad DG8 cartridge (Bio-Rad catalog number: 1864008) in a DG8 Cartridge Holder (Bio-Rad catalog number: 1863051), covered with a DG8 Gasket (Bio-Rad catalog number: 1863009), and then smoothly transferred to the Bio-Rad QX-200 droplet generator (Bio-Rad catalog number: 1864002) which partitions the reaction mix into ~ 20,000 nano-droplets. Each sample's droplets were then pipetted into an individual well of a ddPCR 96-well plate (Bio-Rad catalog number: 12001925). After all droplets were transferred, the plate was sealed using the PX1 PCR plate sealer (Bio-

Rad catalog number:1814000) and then transferred to an MJ Research PTC-100 Thermal Cycler for PCR amplification. Each ddPCR plate run contained field blanks and at least one positive (genomic DNA extracted from the tissue of the target species) and one negative control (containing all reagents except DNA template, which was replaced with DNA-free water). Thermocycling conditions consisted of a 10-minute enzyme activation phase at 95°C followed by 40 cycles of a 30-second denaturation stage at 94°C and a 1-minute Annealing/Extension phase at 60°C. After thermocycling, the samples were subject to a 10-minute enzyme deactivation phase at 98°C followed by a 15-minute droplet stabilization phase at 4°C which was continued indefinitely. The temperature ramp rate was set to 2°C between all steps. Once cycling was complete, the plate was moved to the QX200 droplet reader to estimate the DNA concentrations. Each water sample was analyzed only a single time (i.e., single technical replicate) unless the results showed signs of anomalous fluorescence patterns or low droplet counts. When this occurred, the sample was re-run, and the updated results were used.

Determining limits of detection and quantification

A water sample was considered positive for coho salmon if the estimated DNA concentration was above the limit of detection (LOD). The LOD was defined as the lowest concentration of DNA that would result in at least 95% positive detections which was determined using a probit analysis. The reaction setup consisted of a four-fold serial dilution of coho salmon genomic DNA that ranged in concentration from 3.18 to 0.64 copies per 20µl reaction with 24 replicates per dilution step. Genomic DNA was

extracted from fin clips using a Qiagen DNeasy Blood and Tissue Kit [Qiagen catalog number: 69504] following the manufacturers' instructions. To estimate the LOD, the probit analysis was applied to the proportion of replicates at each dilution step that contained measurable quantities of DNA.

The limit of quantification (LOQ) is the lowest concentration of DNA copies per reaction that could be obtained with a coefficient of variation (CV) less than 20% among replicates. The LOQ reaction setup consisted of eight dilution steps that ranged in concentration from 0.64 – 12,450 copies per 20 µl reaction. When determining the LOQ, I fit a series of models to the CV at each dilution and compared models using Akaike's information criterion (AIC) and the model weights. The set of models consisted of a generalized linear model (GLM), second, third, and fourth-order polynomials, and a generalized additive model (GAM) from package mgcv (Wood 2011). I selected the model with the lowest AIC value to estimate the concentration that would achieve the desired CV of 20% among replicates. These methods are similar to those of Klymus et al. (2020). All models were fit using scripts and functions in the R programming language (R Core Team 2021).

<center>Occupancy analysis</center>

Multi-scale occupancy models and maximum-likelihood estimation were used to compare the detection probabilities of eDNA methods and UVC surveys. Water samples

with an eDNA concentration above the LOD were considered a detection. For my

analysis, I combined field observations from the 2020-2021 surveys.

The parameters of the models were defined as:

$\Psi = $ Pr (Occurrence in a reach)

$\theta_t = $ Pr (Occurrence in survey pool t | reach is occupied)

$p_{m,t} = $ Pr (Detection by survey method m at pool t | reach is occupied, and the species is

  present at the survey pool)

Psi (Ψ) is the probability of species occurrence in a river reach. The next hierarchical

level, theta (θ_t), describes the probability of the species occurrence in any given subunit t

(i.e., pool) of the larger survey reach which is conditional on the species being present

within the reach. Finally, $p_{m,t}$ describes the probability of the species being detected by a

given survey method m in subunit t of the larger survey reach, conditional upon the

species being present in both the reach and the subunit. An example detection history for

a survey pool could be 11101, with the first two numbers representing detections from

each of the two dive passes and the last three numbers representing detections from the

three eDNA samples. In this example, coho salmon were detected at the survey pool by

both divers and in water samples one and three. A detection history in another pool could

be 00000, indicating that neither method detected coho salmon. Note that this

parameterization differs from some other applications of hierarchical modeling of eDNA

in which p for eDNA is defined as the probability of detecting coho salmon DNA in a

replicate qPCR run within a single water sample and θ is the probability that the water sample contains coho salmon DNA (Schmidt et al. 2013; Schmelzle and Kinziger 2016; Dorazio and Erickson 2017; Spence et al. 2021). When using ddPCR, the replicate error can be determined from a single reaction ("Droplet Digital PCR: Applications Guide"). Therefore, I defined p for eDNA as the combined probability of capturing and detecting coho salmon DNA in a replicate water sample and θ is the probability that a survey pool contains coho salmon, similar to Smith and Goldberg (2020). This parameterization allows comparison of the method-specific detection probabilities at the level of the survey pool within the current software limitation of three hierarchical levels.

Multi-method occupancy models were fitted using the multi-method parameterizations available in Program PRESENCE (version 2.13.10; Hines 2006)) to estimate method-specific detection probabilities. This analysis included the 96 pools surveyed with both eDNA methods and the double independent UVC dives. Covariates hypothesized to influence detection probability of the two methods were evaluated. No covariates were included for Ψ or θ because the focus was to compare detection probabilities rather than determine species occupancy patterns and because of the preferential selection of previously occupied reaches in 2021. I hypothesized that UVC detection probability would be reduced by increasing RD and LWD due to difficulties in observing individuals in deeper water or with visual obstructions (e.g., Thurow et al. 2006), and that eDNA detection probability would be reduced by increasing BA due to the dilution of rare eDNA particles (Baldigo et al. 2017). Both RD and BA were $\log_{10}$

transformed. Finally, a covariate for year was included to account for possible differences in detection probabilities between years. Turbidity was not included as a covariate because it was consistently very low, and it was not expected to have inhibited a diver's ability to detect a target.

Occupancy models with different covariate combinations were fitted and ranked using Akaike's information criterion (AIC), ΔAIC, and AIC weights (Burnam and Anderson 2002). With the exception of the null model where the detection probabilities were constant, all models included method as a covariate and then all other possible combinations were developed with each of the other covariates (BA, RD, LWD, year). Covariates were included individually and with interactions by method (with the exception of a year-method interaction). The model ranks remained the same even when using a small sample size correction for AIC (AIC$_C$) with the number of survey locations ($n = 96$) as the effective sample size; however, I chose to present the AIC values because there is currently no consensus on the best way to calculate AIC$_C$ for multi-scale occupancy models (MacKenzie et al. 2018).

After determining the best occupancy model from the set of considered models, I used the estimated coefficients and their variance-covariance matrix (obtained from PRESENCE) to generate response plots of the effect of each covariate on the detection probabilities. Predictions were made over the observed range of values for a covariate while all other covariates were held at their median value. A Monte Carlo approach was used to approximate the standard error (SE) for the estimated detection probabilities. This

was done by taking 1000 random samples of coefficients from a multivariate normal

distribution defined by the estimated coefficients and their variance-covariance matrix

using the MASS package in R version 4.0.5 (Venables and Ripley 2002; R Core Team

2021). Each set of coefficients was used to generate a response curve for each covariate

(while holding the other covariates at their medians). The approximate SE for the

response plots was calculated as the middle 69% of the 1000 Monte Carlo predictions

that were generated for each covariate value.

I also calculated the cumulative probability of detecting coho salmon DNA, as a

function of the number of replicate water samples (n) taken from a pool that contained

coho salmon DNA. The cumulative probability of detection (p^*) was calculated using the

equation $p^* = 1 - (1 - p_t)^n$. This calculation was done using the highest, median, and

lowest p_t estimated for the sampled pools based on the observed covariates and the best

occupancy model. These cumulative detection probabilities indicate the required

sampling effort needed to detect coho salmon DNA with a specified probability under

several scenarios (McArdle 1990).

Concentration-count analysis

For objective two, I assessed if the observed eDNA concentrations and covariates

in a pool could be used to predict the within-pool fish counts. To explore this

relationship, I used a zero-altered (ZA) model (i.e., a hurdle or delta model). The ZA

model is a two-part model in which a binomial model is used to assess the probability of

getting a zero count, and a zero-truncated model (with a negative binomial distribution in this application) is used to model the positive, non-zero data, while accounting for overdispersion (Zuur et al. 2009). The probability of getting a non-zero is multiplied by the estimated count from the zero-truncated count model to predict the overall count for the "full" model (Zuur et al. 2009). The ZA model was fit using package glmmTMB (Brooks et al. 2017). The response variable was the average of the two counts from the double-pass survey pools (rounded to the nearest integer). The predictors for both the binomial and count parts of the model were the average of the three eDNA concentrations that were transformed using the natural logarithm (hereafter ln(eDNA)), LWD, RD, and BA. An offset of the natural log of pool area (hereafter pool area) was also included to account for variation in pool size, where pool area was calculated as the product of the max pool length and the representative average pool width. Due to the limited number of data points (n = 91) caused by missing covariate data and the relatively low number of non-zero eDNA observations, all eDNA concentrations were used as estimated, regardless of whether the concentrations were below the LOD (n = 4) or LOQ (n = 15).

To assess the effect of ln(eDNA) and habitat covariates on both the binomial and count portions of the model, I compared models with all possible covariate combinations using Akaike's information criterion with corrections for small sample size with package MuMIn (Bartoń 2020). The combined binomial and count models were restricted to have no more than six total coefficients (not including an intercept), to prevent overparameterization of models fit to a data set with n = 91. Any models that failed to converge were omitted from consideration and the ΔAICc values and model weights

were recalculated for the remaining models. I used the top model of the resulting set to estimate the effects of the covariates on the binomial, count, and combined models while holding all other covariates at their median values.

RESULTS

Limits of detection and quantification

I found that the LOD for the coho salmon assay was 6.56 copies of DNA per reaction and the LOQ was 46.77 copies per reaction. Because the dilution range used in the probit analysis did not encompass the LOD, I extrapolated from the observed data to obtain the LOD of 6.56 copies per 20 µl reaction (Figure 3). However, this estimate has since been confirmed by several independent in-house projects (Gavin Bandy, Cal Poly Humboldt, personal communication; Braden Herman, Cal Poly Humboldt, personal communication). The LOD threshold was rounded to 7 copies of DNA per 20 µl reaction for more conservative estimates, resulting in 32 pools with detectable levels of DNA (out of 96 total pools). Reducing the LOD by half (to 3.5) identified only two additional samples as detections, but these were from pools that already had detections and thus would not have impacted the number of pools estimated to be occupied with the eDNA methods. For determining the LOQ, a third-order polynomial was the best model (Table 1) and indicated that a concentration of 46.77 copies per 20 µl reaction would achieve the desired level of allowable variation (i.e., 20% CV) between replicates (Figure 3).

(A)

(B)
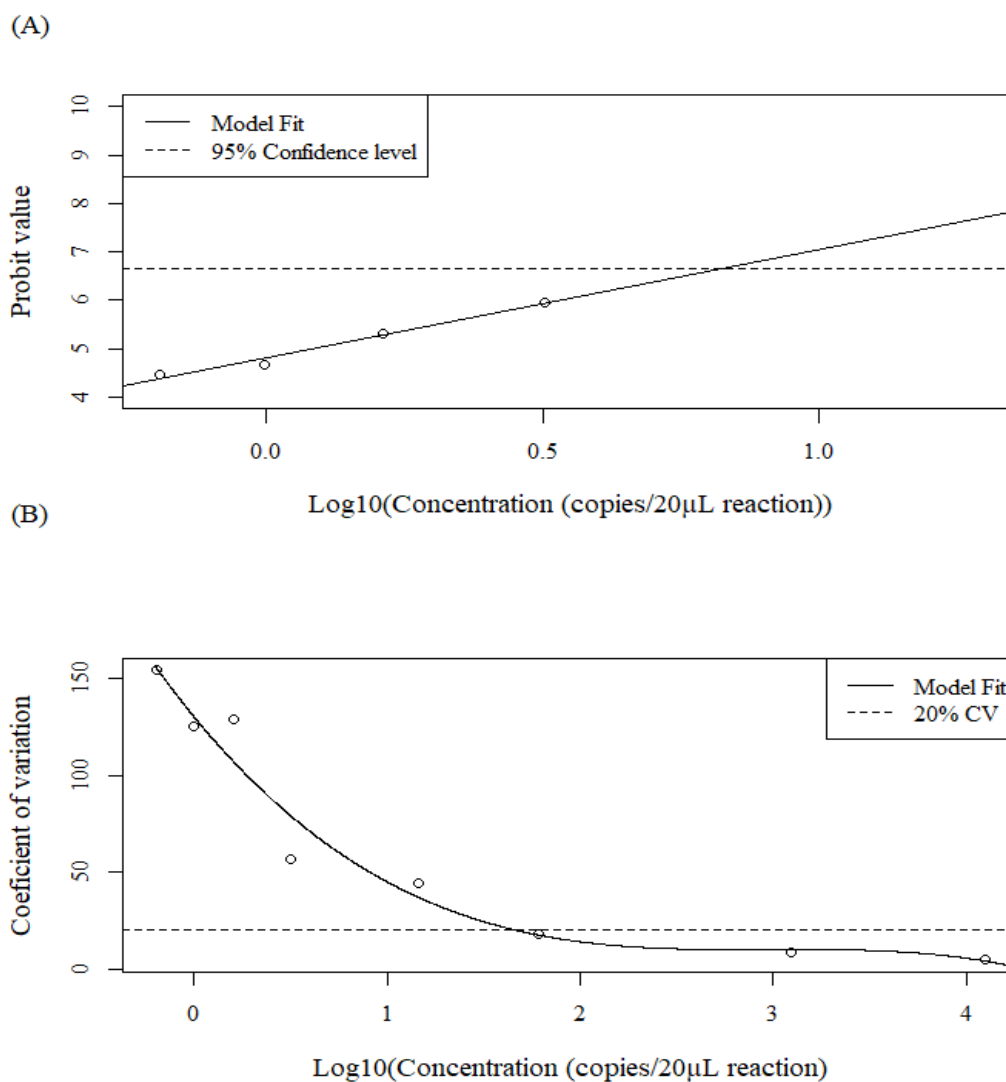
Figure 3. Results of (A) the probit analysis for determining the Limit of Detection (LOD) and (B) the predictions of the best model for determining the Limit of Quantification (LOQ; B). (A) Points represent values from a dilution series, solid lines represent best model fits, and dashed lines represent a 95% confidence level (A) and the 20% coefficient of variation (CV) threshold.

Table 1. Model selection table for selecting the best model to determine the limit of quantification. The AIC values, ΔAIC, and the model weights (AIC Weights) were used to determine the best model.

Model	AIC	ΔAIC	AIC Weight
Third-order polynomial	71.77	0	0.37
GAM	72.20	0.43	0.30
Second-order polynomial	73.06	1.28	0.19
Fourth-order polynomial	73.70	1.93	0.14
Linear	82.16	10.4	0.00

Survey results

A total of 96 pools distributed among 25 reaches were surveyed using both eDNA and UVC methods in 2020 and 2021. An additional 318 pools were surveyed with UVC methods only. Coho salmon were detected in six of the 19 reaches surveyed in 2020 and all seven reaches surveyed in 2021. In reaches where coho salmon were observed, counts ranged from 0-210 fish per pool with an average of 33 individuals across all pools (Figure 4A). The average difference between the two independent dive counts was 4 individuals or 34% across all pools. Reaches were on average two kilometers in length. LWD counts ranged from 0-11 structures per pool, RD ranged from 1-320 cm, and BA ranged from 0.26-155 km^2 per reach (Figure 4). None of the field blanks or negative internal controls tested positive for coho salmon DNA.
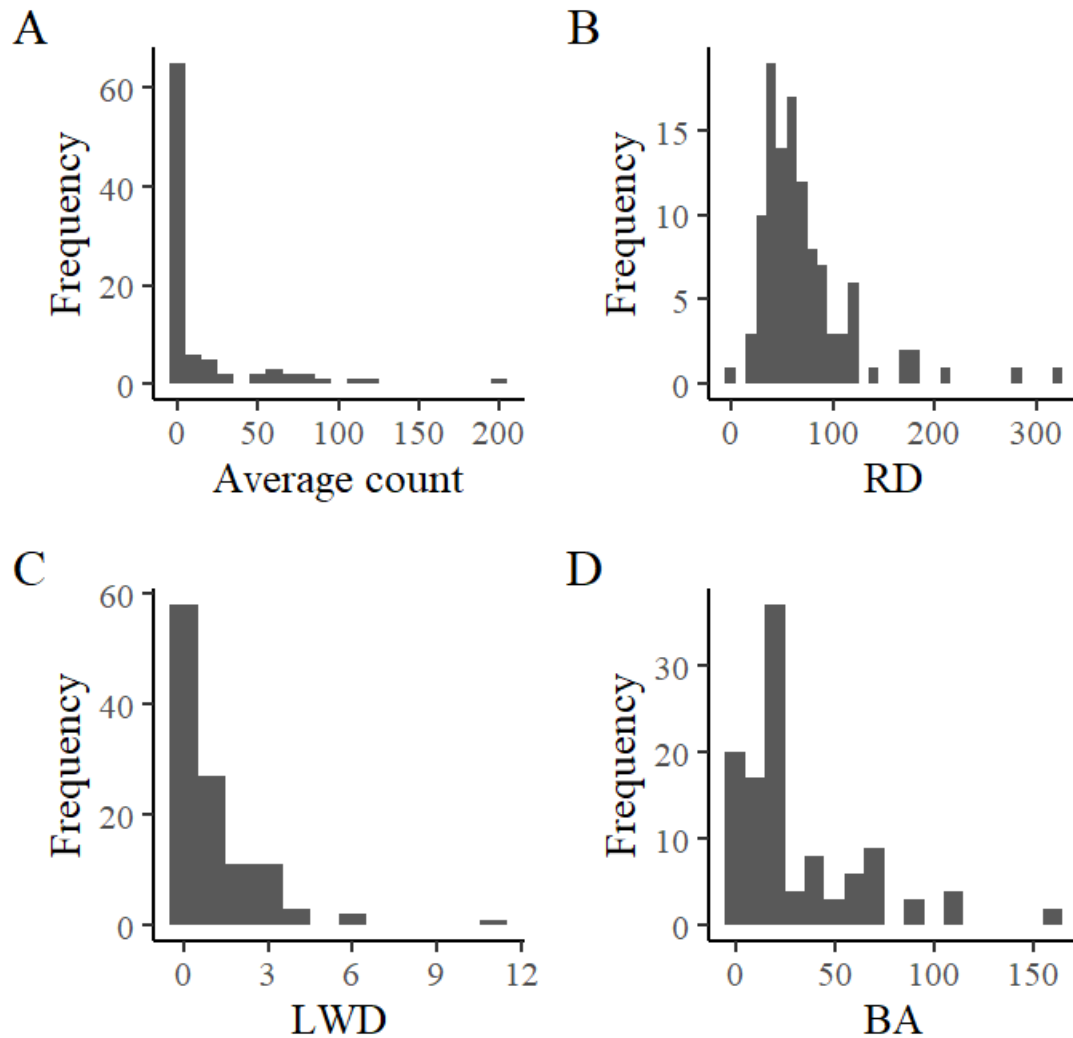
Figure 4. Frequency distributions of (A) the average count per pool of coho salmon, (B) the Residual Depth (RD in cm), (C) Large Woody Debris (LWD), and (D) Basin Area (BA in km²) observed in the 2020-2021 survey seasons.

Among the 96 pools surveyed using both UVC and eDNA methods, coho salmon were detected in a total of 29 pools by UVC and in 32 pools using eDNA methods (Table 2). Both methods detected coho salmon at 28% of pools (n_{pool}=27) and neither method detected at 65% of pools (n_{pool}=62), indicating that the two methods had agreement with

regards to detection at 93% of surveyed pools (n_{pool}=89). There was disagreement

between the methods at only 7% of all survey pools (n_{pool}=7). At 2% of survey pools

(n_{pool}=2), coho salmon were detected by UVC but not by eDNA, whereas at 5% of survey

pools (n_{pool}=5), coho salmon were detected by eDNA but not by UVC surveys (Table 2).

When comparing methods at the level of the survey reach using only the double-

pass survey pools, eDNA detected coho salmon in one more reach than UVC (Table 2).

There were eDNA detections in three reaches where no coho salmon were observed, and

there were two reaches where no eDNA was detected but coho salmon were observed.

The two methods agreed at 80% of reaches (n_{reach} = 20) with detections at 24% (n_{reach} =

6) and non-detections at 56% (n_{reach} = 14). However, if the 316 intermediate UVC survey

pools were included, thus changing the total sample size for UVC (n_{pool} = 414) relative to

eDNA (n_{pool} = 96), then coho salmon were observed by UVC in two additional reaches

where they had not been observed in a double-pass pool. In one of these instances, coho

salmon had only been detected with eDNA methods, while the other was in a reach that

previously had no detections by either method. Inclusion of the broader UVC results did

not alter the overall agreement (80%) at the reach scale (Table 2).

Table 2. The percentage of survey pools and reaches in which coho salmon were detected (+) or not detected (-) by each survey method (i.e., eDNA and UVC). Numbers in parentheses indicate the number of pools or reaches. Pool comparisons are based on the 96 double-pass pools that were surveyed using both methods. Reach comparisons were calculated for the 25 reaches using either the 96 double pass pools or using an additional 318 pools where only UVC observations occurred.

eDNA Detection	UVC Detection	Pools	Reaches (based on double-pass pools)	Reaches (based on all pools)
+	+	28% (27)	24% (6)	28% (7)
-	-	65% (62)	56% (14)	52% (13)
+	-	5% (5)	12% (3)	8% (2)
-	+	2% (2)	8% (2)	12% (3)

Occupancy results

Of the 23 occupancy models examined, five had a ΔAIC less than two with strong evidence of variation in detection probability by survey method. All five models indicated that detection probabilities varied by survey method, RD, and BA, but the top model (AIC Weight = 0.25) also included a method-BA interaction (Table 3). The estimated occupancy probability from the top model over the combined survey years was $\psi = 0.48$ (SE: 0.10, 95% CI: 0.29 - 0.67) and a conditional probability of occurrence in a pool was $\theta = 0.78$ (SE: 0.06, 95% CI: 0.64 - 0.87).

*Table 3. Top five occupancy models (with ΔAIC < 2) for juvenile coho salmon. Psi represents the probability of occupancy within a reach, theta is the probability of occurrence within a pool given that a reach is occupied, and p is the detection probability given that a pool is occupied. Parameters were modeled as a constant (.), or as a function of survey method (m), count of large woody debris (LWD), residual pool depth (RD), contributing basin area (BA) or an interaction between a habitat covariate and method (e.g., BA * m). K represents the number of estimated parameters in the model. Differences in AIC values relative to the top-ranked model (ΔAIC) and model weights (AIC Weight) are provided for all models.*

Rank	Model	K	ΔAIC	AIC Weight
1	psi(.), theta(.), p(m, RD, BA, BA*m)	7	0	0.251
2	psi(.), theta(.), p(m, RD, BA)	6	1.48	0.120
3	psi(.), theta(.), p(m, LWD, RD, BA, LWD*m, RD*m, BA*m)	10	1.84	0.100
4	psi(.), theta(.), p(m, RD, BA, RD*m, BA*m)	8	1.89	0.098
5	psi(.), theta(.), p(m, RD, BA, Year, BA*m)	8	1.95	0.095

The method-specific detection probabilities for the best model were similar under median conditions and were similarly affected by RD; however, BA had a stronger negative effect on *p* for eDNA methods than UVC surveys (Figure 5). Under median environmental conditions, the estimated detection probability for eDNA was $p_{eDNA} = 0.91$ (SE: 0.04, 95% CI: 0.80 - 0.96), and for UVC it was $p_{UVC} = 0.89$ (SE: 0.03, 95% CI: 0.79 - 0.94). The detection probabilities for both methods increased with increasing RD, with predicted p_{UVC} ranging from 0.61 – 0.94 and p_{eDNA} from 0.68 – 0.96. Increasing BA had a strong negative influence on the detection probability of eDNA, but mostly at values of log(BA) greater than one, at which point detection probabilities decreased rapidly from

0.99 to 0.13 (Figure 5B). The detection probability of UVC was also slightly negatively associated with BA with p_{UVC} decreasing from 0.98 to 0.71), but the effect was not as pronounced as with eDNA (Figure 5B).
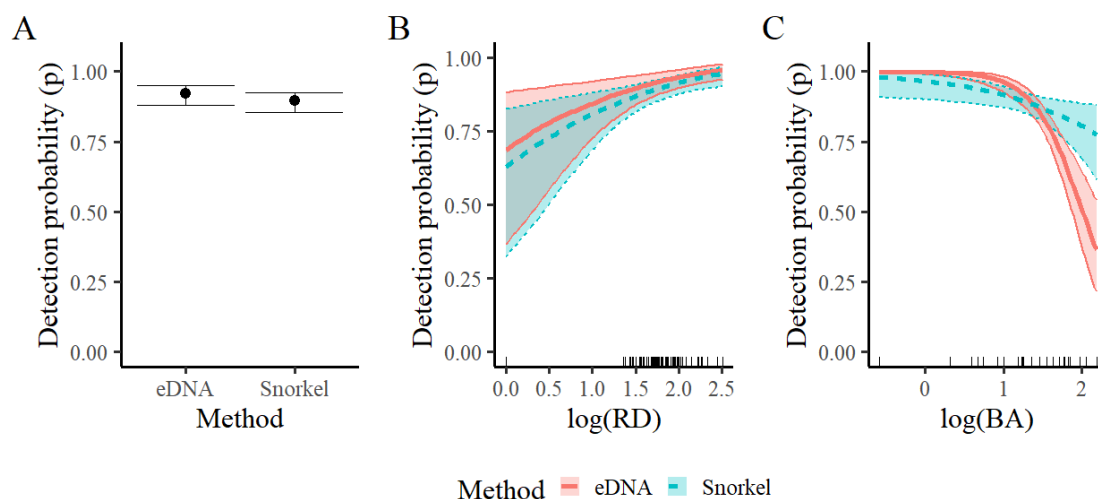
Figure 5. Predicted effects of (A) method, (B) log of residual pool depth (RD), and (C) log of basin area (BA) on detection probabilities for eDNA (thick solid line) and UVC (thick dashed line) with the associated standard error. Effect sizes were calculated over the observed range of values for the covariate (ticks) while all other covariates were held at their median values. SE of the estimates (bands) was approximated using the middle 69% of predictions from 1000 Monte Carlo simulations.

The estimated pool-specific eDNA detection probability ranged from p_t= 0.13 - 0.99 for a single replicate water sample and single technical ddPCR replicate based on the observed range of covariate values at each pool. Given the presence of DNA in a survey pool with median values of RD and BA, there was a high probability (p^*=0.91, 95% CI = 0.79 – 0.95) of capturing and detecting coho salmon DNA with one water sample (Figure 6); only two replicate water samples would be needed to have a >95% cumulative detection probability for capturing and detecting coho salmon DNA under the median environmental conditions. At reaches where detection probabilities were

estimated to be the highest (i.e., $p_t = 0.99$), only a single water sample would be required to surpass the 95% cumulative detection probability mark. However, 21 water samples would be needed to achieve a cumulative detection probability of 95% (with a high degree of uncertainty) at locations with the lowest estimated eDNA detection probabilities (i.e., $p_t = 0.13$; at a pool with the lowest RD [1 cm] and second highest BA [114 km^2]).
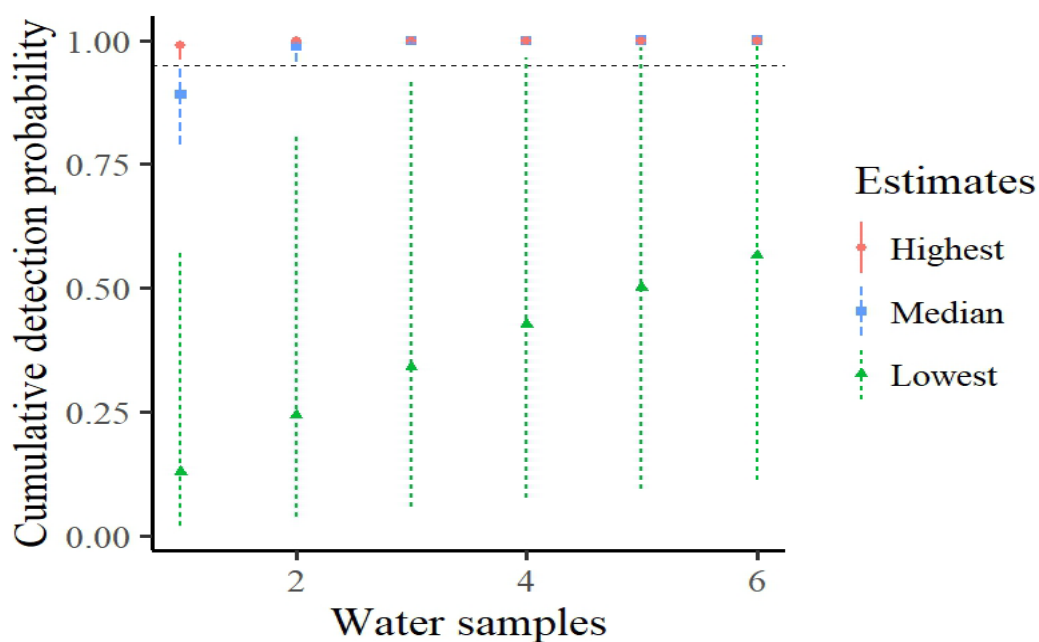
Figure 6. The cumulative detection probability for the highest (p = 0.99), median (p = 0.89), and lowest (p = 0.13) pool-specific detection probabilities calculated for different numbers of replicate water samples. The vertical bars represent the 95% confidence interval. The horizontal dashed line represents the 95% probability of detection given the presence of coho salmon DNA in the survey pool.

Concentration-count analysis

For objective two, I used ZA models to assess the utility of using the observed eDNA concentrations to predict the counts in the survey pools. The model selection

procedure indicated that the top model included the log(BA) and pool area offset for the count model, while LWD, ln(eDNA), RD, and the offset of pool area were included in the binomial model (Appendix A, Table A1). Basin area, which was only included in the count model, had a negative influence on the expected counts as was hypothesized; however, there was a large amount of uncertainty in the estimated counts at low BA values (Figure 7A). After accounting for the probability of a zero count in a pool, predicted counts are much lower across all pools and the effect of BA on counts is less apparent (Figure 7B).
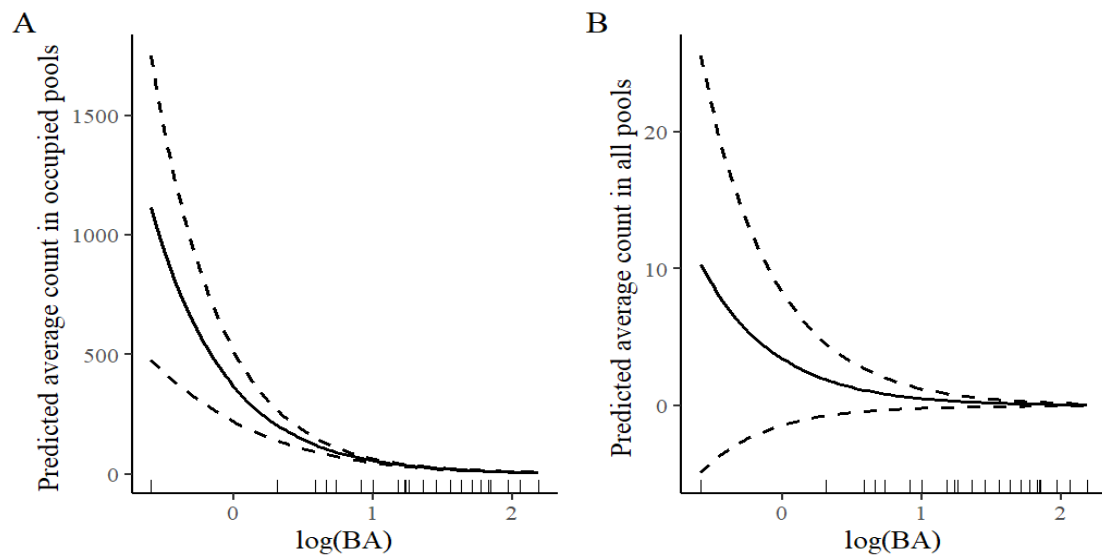
Figure 7. The predicted effect of increasing basin area (BA) on the average count of coho salmon in a pool. The predictions (solid line) and their standard errors (dashed lines) are shown for (A) the count model and (B) the full zero-altered model while all other covariates are held at their median values. Estimates are based on the observed data (ticks). The count model predicts the mean number of fish present in occupied pools whereas the full model predicts the mean number of fish in all pools (occupied or not).

Pool area, which was included as an offset to account for the size of the survey pools, was the only covariate to be included in both the count and binomial parts of the ZA model (Figure 8). The count model indicates that as the size of a pool increases it is expected to hold more coho salmon, given that the pool is occupied. However, the binomial model indicates that the probability of a coho salmon occurring in a survey pool decreases from 0.16 to approximately zero over the observed range of pool areas, though there is a large amount of uncertainty at low values of pool area. The effects of these two models oppose each other which explains the relatively small effect shown in the full model with the 95% confidence interval overlapping zero (Figure 8).

In the binomial part of the ZA model, eDNA concentration, LWD, and RD were included as predictors of the presence or absence of coho salmon in a survey pool, but only eDNA had a strong effect. The predicted counts as a function of the eDNA concentration had sigmoidal shapes for both the binomial and full ZA models (Figure 9). For the binomial model, the probability of occurrence was approximately zero at low concentrations of DNA, however as the concentration of DNA increased in a pool the probability that the pool is also occupied by coho salmon increased. The predicted count from the full ZA model had an asymptote at the average number of observed coho salmon (i.e., 33 individuals). This analysis did not use the LOD or LOQ that was estimated for the eDNA assay and was independent of the LOD and LOQ estimates. However, results indicated that the concentration that achieved 50% probability of presence was nearly identical to the LOD (Figure 9A), and the LOQ corresponded with concentrations where the standard errors for the binomial model were extremely small

(Figure 9B). The binomial model indicated that LWD and RD both had slightly negative

effects on the probability of coho occurrence, although the effect sizes were small, and

the 95% confidence intervals included zero at nearly all observed values of the covariates

(Figure 9). Overall, both LWD and RD were predicted to have negligible effects on the

predicted count in the full model with relatively high levels of uncertainty.
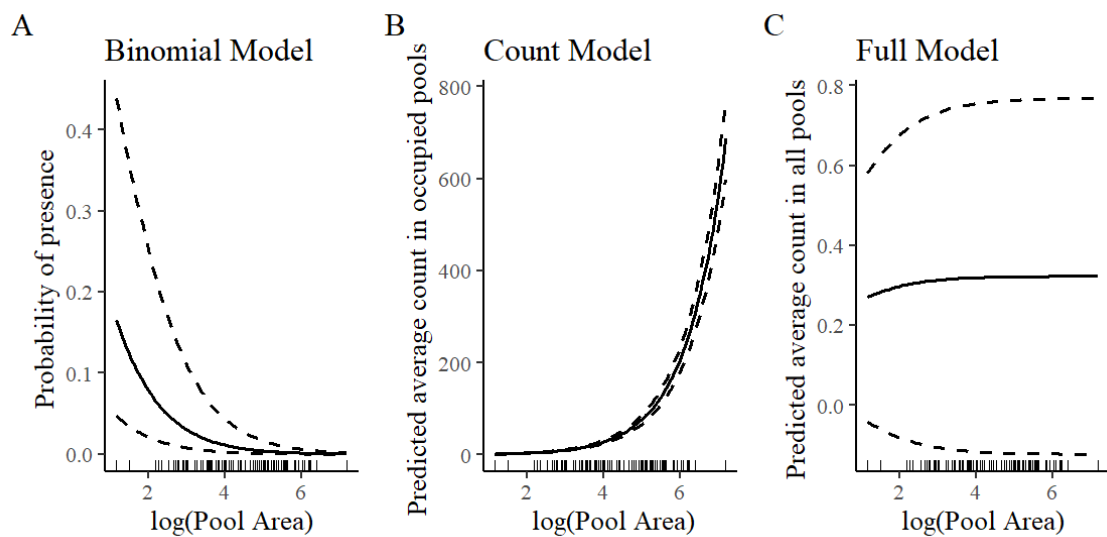
Figure 8. The predicted effect of increasing pool area on the average count and probability of coho salmon being present in a survey pool. The predictions (solid lines) for the (A) binomial, (B) count, and (C) full zero-altered model with their standard errors (dashed lines) are shown. Ticks on the X-axis represent values for the observed data.
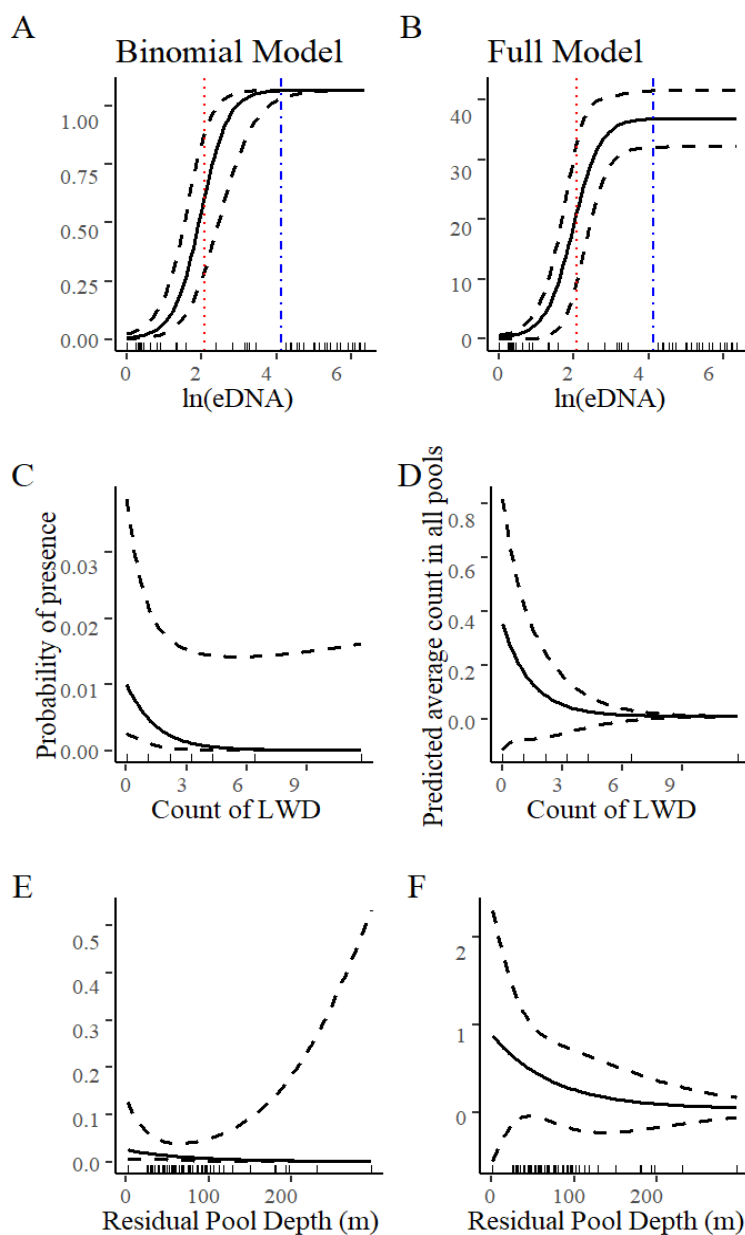
Figure 9. The predicted effect of mean ln(eDNA concentration) (panels A and B), large woody debris (LWD; panels C and D), and residual pool depth (RD; panels E and F). The predictions (solid lines) and their standard errors (dashed lines) are shown for the binomial model (panels A, C, and E) and the full zero-altered model (panels B, D, and F) while all other covariates are held at their median values. Estimates are based on the range of observed data (ticks). The estimated limit of detection (LOD; dotted red vertical line) and limit of quantification (LOQ; alternating dotted and dashed blue vertical line) are shown in panels A and B.

DISCUSSION

This study adds to the limited body of literature on the comparison of the method-specific detection probabilities of eDNA and conventional surveys in freshwater river systems (Fediajevaite et al. 2021). Furthermore, results corroborate the findings of other studies which suggest that eDNA is a highly sensitive method for surveying rare species (McKelvey et al. 2016; Rice et al. 2018; Strickland and Roberts 2019; Sutter and Kinziger 2019). The detection probability of eDNA in this study was high and equivalent to that of conventional UVC surveys for coho salmon in all but the largest basins in the study system. These findings suggest that eDNA methods could be a viable alternative to UVC surveys when establishing species occupancy in systems like the Smith River. However, these results do not support the use of eDNA as a replacement for UVC surveys in this system as eDNA could not be used to predict the average count of coho salmon in a survey pool.

Detections

The high level of agreement in detections at the scale of survey pools (93%) and reaches (80%) indicated that both eDNA and UVC methods were comparable in their basic ability to determine species distribution patterns. By comparing the survey methods at only the double-dive pools, eDNA alone detected coho salmon on more occasions (i.e., five double-pass pools and three reaches) than UVC methods alone (i.e., two double-pass pools and two reaches). If, however, the additional 316 intermediate pools were included,

thus increasing the total UVC pool sample size to over three times that of eDNA, then UVC had slightly more reach level detections than eDNA methods. These results demonstrate that eDNA was able to achieve similar estimates of coho salmon spatial distribution with less overall effort, which has been noted in other studies (Evans et al. 2017; Yu et al. 2021).

The higher level of agreement (93%) between eDNA and UVC surveys at the pool scale relative to the reach scale (80%) was unexpected as previous studies have shown greater agreement between methods at large scales (e.g., a watershed or river reach) than small scales (e.g., pools; Castañeda et al. 2020; Spence et al. 2021). This difference could be attributed to the differences in sample sizes used for these comparisons and random chance; observed agreement at the scale of reaches was more sensitive to single detections within a reach because fewer reaches were surveyed. For example, there were two cases where coho salmon were detected in a reach by only one method in a single pool which decreased the reach level agreement.

Occupancy modeling

Occupancy modeling indicated that both methods had similarly high probabilities of detecting coho salmon in a pool and thus are highly effective survey methods with detection probabilities of 89% for UVC and 91% for eDNA at median values of covariates. These detection probabilities are higher than those reported in other eDNA studies (Matter et al. 2018; Akre et al. 2019; Smith and Goldberg 2020; Castañeda et al. 2020; Spence et al. 2021) as well as in a meta-analysis that compared eDNA methods to

conventional aquatic and terrestrial survey methods for numerous taxa (Fediajevaite et al. 2021). Fediajevaite et al. (2021) found that the median detection probability of eDNA methods was $p\sim0.80$, and that it was significantly higher than the median detection probability of conventional methods ($p\sim0.68$). Additionally, Fediajevaite et al. (2021) indicated that eDNA methods were most often reported as more sensitive, cost-effective, and able to detect a wider variety of species than conventional methods (Fediajevaite et al. 2021). However, the authors note that these findings could be affected by publication bias in which studies with greater differences are published more readily.

Variability in detection probabilities among eDNA studies could be linked to differences related to eDNA processing. Factors related to eDNA processing could include in-field or laboratory filtration and preservation (Majaneva et al. 2018), volume of water filtered (Capo et al. 2020), the effects of inhibition (Jane et al. 2015), filter pore sizes (Turner et al. 2014; Jo et al. 2021), and the method of analysis (i.e., PCR, qPCR, ddPCR). In this study, all samples were filtered and preserved in the field using a filtration manifold and cell-lysis buffer solution which has been shown to produce more consistent yields of eDNA than other methods (Williams et al. 2016; Kumar et al. 2020; Mauvisseau et al. 2021). Additionally ddPCR has been shown in other studies to be more sensitive than PCR and qPCR methods with higher detection rates (Doi et al. 2015a, 2015b; Hamaguchi et al. 2018; Uthicke et al. 2018; Brys et al. 2020; Wood et al. 2019). Finally, the relative insensitivity of ddPCR to environmental inhibitors permits the use of large volumes of analyte (i.e., maximum of 15 μl), theoretically increasing the probability of target DNA being present in the sample due to the increased volume (Rees et al. 2014;

Doi et al. 2015a; Te et al. 2015; Mauvisseau et al. 2019). I did not test the effects of

ddPCR sample volume on detection rates, but I echo the recommendations of Doi et al.

(2015) that future studies should examine the change in ddPCR detection rates at low

DNA concentrations by increasing PCR sample volumes.

While both methods in this study had high detection probabilities, the top

occupancy model indicated that detection probabilities were strongly influenced by BA.

The results showed that the detection probabilities of both methods decreased with BA

but that eDNA was more strongly affected by large BA. At the lowest (i.e., 0.26 km^2) and

median (i.e., 23 km^2) values of BA, only one to two water samples were required to have

95% cumulative detection probability in a survey pool, but up to 21 water samples would

be needed at the highest (i.e., 155 km^2) BA. These results are consistent with the

hypothesis that increasing discharge, for which BA is a proxy, would decrease the

probability of capturing and detecting rare organismal DNA due to the dilution of

particles, and this has been shown in other studies as well (Wilcox et al. 2016; Baldigo et

al. 2017; Spence et al. 2021). The negative effect of BA on UVC detection probability

could be due to challenges in surveying with higher discharge (which was also correlated

with pool area). The negative BA effect for eDNA may have been more substantial

because the eDNA sampling effort per pool was fixed in this study (three samples per

pool), whereas the sampling effort for UVC (in terms of area surveyed) was

commensurate with pool size. Additional work is needed to explore how the probability

of capturing eDNA in a water sample changes with regard to the overall discharge of a

study system and the abundance of a target organism. Future studies should consider

altering sampling effort (e.g., number of samples, larger sample volumes) with basin size when attempting to detect coho salmon or other rare species using eDNA methods.

The RD was an important covariate for detection probability, but its effect was small and strongly influenced by a single outlier that resulted in large SE of predictions at low RD values. The RD was the measure of pool depth that was collected by the UVC crew because it is a more consistent approximation of relative pool depth over time as measurements are independent of discharge (Lisle 1987). Generally, survey pools with low values of RD are more similar to runs or riffles, and higher values of RD indicate a more "pool like" unit. Therefore, units with low values of RD are more likely to have faster water velocities and can be shallower, making it more difficult for a UVC observer to survey a pool and reducing the UVC detection probabilities. The low eDNA detection probabilities in pools with low RD may have resulted from dilution caused by faster moving water or perhaps from insufficient mixing of DNA particles in the water column within a pool. Although the effects of RD were small across the majority of the sampled pools (excluding the outlier with a low RD), future eDNA studies should record actual pool depth at the deepest point and water velocity to explore the hypotheses more directly.

The count of LWD was hypothesized to account for visual obstructions within a survey unit, however it was not included as an important covariate in the top model. This may suggest that coho salmon were relatively easy to detect even with high amounts of visual obstructions. Another potential explanation is that LWD may have been an

insufficient proxy of the number of visual obstructions within a pool which can also include undercut banks and large substrate (Thurow et al. 2006). For future surveys, I recommend that surveyors measure the amount of cover (e.g., log jams, undercut banks, roots, complex rocky substrate) within the pool that may obstruct the divers' vision.

<div align="center">Transport of eDNA and modeling assumptions</div>

In river systems, eDNA is transported away from the source organism by the downstream flow of water, and an upstream target could be detected at multiple downstream points if the eDNA is transported long distances and remains at detectable levels (Goldberg et al. 2016). Although this transport can be highly beneficial for establishing the presence of a target species across a larger spatial area in a study system, it is also an important consideration when applying occupancy modeling to eDNA data as neighboring sampling locations within the same system may not be independent and could bias model predictions (MacKenzie et al. 2018)

The downstream transportation of eDNA particles had the potential to violate the independence assumption of the occupancy model, but this was unlikely in this study based on transport scales recently reported in the literature. Studies conducted in similar systems suggest that the spatial scale of eDNA transport under base flow conditions is likely less than the average spacing between my eDNA sampling pools, which were on average 536 m (95% C.I. 438 - 635 m) apart. Jo and Yamanaka (2022) conducted a meta-analysis of nine eDNA studies and found that the average distance eDNA particles were transported downstream was 218 m (95% C.I. 112 - 425 m). Spence et al. (2021) found

that eDNA from low numbers of coho salmon (n = 15) were most commonly detected between 10 and 50 m downstream of the source and were very rarely detected at distances past 200 m during low summer baseflow conditions in coastal rivers of central California. Another ongoing study in coastal streams and rivers of Northern California has found that foreign eDNA introduced at very high concentrations is most commonly detected within 450 m of the source (Braden Herman, Cal Poly Humboldt, personal communication). These results suggest that the spacing of the sampling pools was sufficient for the assumption of independence to be satisfied in the occupancy model.

An additional, related concern was that some eDNA detections could have resulted from fish that were not in the survey pool but were just upstream. This would have caused an unfair comparison between the two methods as coho salmon would not have been available in the survey pool for UVC observers to detect. Three lines of evidence suggest that this was not a major concern in this study. First, when fish were not detected by UVC in a double-pass pool it was unlikely that they would be detected in an upstream single-pass pool. There were only two instances where coho salmon were not observed in double pass pool but were observed in a following upstream single-pass pool. Second, there were few cases where eDNA detected fish and UVC did not (5 pools, 5% of samples), and only one of these instances could be potentially explained by fish observed in one of the three immediate upstream single-pass pools. But even in this singular instance, three coho salmon were observed ~300 m upstream of the sampled double-pass pool, and Spence et al. (2021) suggest it is unlikely that such few individuals could have been detected ~300 m downstream. Finally, modifying the analysis to account

for the potential effects of upstream fish in the single-pass pools would not have altered the findings substantially. I considered modifying the occupancy model to estimate method-specific detection probabilities across a sub-reach (i.e., a double-pass and three upstream single-pass pools) rather than at a survey pool, but the results would have remained largely the same with the exception of the one unit described above. I acknowledge that it is possible that fish could be present upstream (e.g., in an unsampled pool), and in the present analysis, these seemingly rare situations would be attributed to divers failing to observe the individuals in the sampled pool. Overall, these results suggest that the comparison of the two methods at the level of the survey pool was a fair comparison and that the downstream transportation of eDNA particles did not greatly influence the eDNA detection probability in this study. However, future studies should be cognizant of these challenges and adjust their sampling design or analytical methods accordingly for their specific applications.

Another concern regarding the occupancy modeling was that including covariates for reach-level occupancy (psi) or pool-level occupancy (theta) could alter our findings for detection probability. To test for these effects, several additional models were fitted with covariates for psi (BA and year) and for theta (RD and LWD). The addition of these covariates had a negligible effect on the estimated coefficients for the detection probability terms (with only a ~0.02% change), indicating that our conclusions for detection probability were not sensitive to such changes.

Concentration-count analysis

Several studies have shown that eDNA concentrations can be strongly related to indices of species abundance, but practical applications in the field have yielded inconsistent results (reviewed in Yates et al. 2019). There was not a significant relationship between the average count of coho salmon and the eDNA concentrations in the present study. Adding a random effect of reach in the GLM did not affect this finding, and instead it is likely due to the relatively small differences in average observed counts among survey pools (i.e., 0-250 individuals per pool, with a mean of 33 fish). Studies that have identified eDNA concentration as a good predictor of abundance indices have typically been in settings with substantially higher variability and contrast in fish abundance, biomass, or density (Yates et al. 2019; Sepulveda et al. 2021). For example, Pochardt et al. (2020) found eDNA concentrations to be correlated to mark-recapture estimates of eulachon (*Thaleichthys pacificus*) abundance when they ranged from zero to 25 million individuals, and Shelton et al. (2022) found that eDNA concentrations to be correlated to acoustic estimates of Pacific hake (*Merluccius productus*) biomass when they ranged from zero to 5,000 tons. When abundances are low, eDNA methods are likely best applied to estimating species occupancy and distribution instead of relative abundance as has been suggested in other studies (Yates et al. 2019; Sepulveda et al. 2021).

Implications for monitoring and management

This study has shown that eDNA surveys could be a suitable alternative or complement to CDFW's standard summer UVC surveys for juvenile coho salmon distribution in the Smith River, but more work is needed to develop a robust and optimal sampling design. The protocol of collecting triplicate 1-liter water samples was sufficient to achieve a 95% probability of detecting coho salmon DNA in a pool (if present) in basins up to 70 km^2, but in larger basins, more eDNA sampling effort would be required to achieve similar confidence levels for detection. Instead of filtering substantially larger volumes of water which can be difficult in some systems (Capo et al. 2020), future eDNA monitoring efforts should consider altering the number of water samples commensurate with basin size to maintain high cumulative detection probabilities. The number of pools to sample in a reach is also an important sampling consideration. Pool-level occupancy by juvenile coho salmon in this system has previously been estimated to be 0.47 (SE 0.02; Walkley and Garwood 2017); at this pool occupancy rate, surveying five pools within a reach for eDNA would yield a >95% cumulative probability of detecting coho salmon at the reach scale. However, additional work is still needed to develop an optimal eDNA sampling protocol for the Smith River survey that balances pool-level occupancy estimates and the downstream transportation of eDNA particles.

The feasibility of implementing eDNA surveys in future monitoring efforts will depend in part on its cost relative to the traditional UVC methods. A low-end cost comparison of the eDNA and UVC methods used in this study indicated that the average

cost per reach for eDNA ($579 per reach) was 18% less than the UVC survey ($707 per reach; Appendix B, Table B1). However, this is only a rough estimate as it did not include the startup and maintenance costs of eDNA laboratory equipment (e.g., laboratory space, ddPCR setup and analysis equipment, etc.), or potential differences in survey effort. Additionally, the costs presented for the eDNA field collections account for only one surveyor as eDNA was added on to the existing UVC survey. Previous studies and experiences in the field suggest that eDNA methods could require less overall survey effort than the UVC survey which would in turn allow for a greater number of sampled reaches per day, further increasing the cost effectiveness of eDNA methods (Wilcox et al. 2016; McKelvey et al. 2016; Evans et al. 2017; Sutter and Kinziger 2019; Fediajevaite et al. 2021).

Results from this study suggest that eDNA methods are not a suitable wholesale replacement for UVC surveys in this system because eDNA methods could not quantify coho salmon counts. However, eDNA methods could be applied in future Smith River surveys to enhance and augment the existing monitoring program for establishing occupancy patterns as the method appears to be more cost effective with a similar detection probability to UVC. One possible scenario would be to utilize both eDNA and UVC methods in an adaptive sampling design. In this scenario, eDNA samples would be rapidly collected by small survey crews from a large number of GRTS reaches using a systematic sampling design. Larger UVC survey crews could return to a subset of reaches where coho salmon eDNA was detected and conduct more extensive multi-pass surveys to obtain robust estimates of abundance within the selected occupied reaches, which

could then be used to estimate the total population abundance. However, additional work is still needed to develop an optimal sampling design that minimizes costs and overall sampling variance while meeting survey objectives.

Fisheries professionals designing future surveys to assess the distribution or occupancy of rare aquatic species should consider the use of eDNA methods as a potential alternative or complement to conventional survey methods. However, this decision should be influenced by several factors. First, future studies should consider the specific study objectives because eDNA may not be suitable for all applications, such as estimating relative abundance or detecting rare species occupying large rivers. Second, potential users should consider any potential tradeoffs between detection probabilities and survey cost on a case-by-case basis when comparing methods. For example, higher detection probabilities for one method could offset its higher costs if one method isn't clearly favored in terms of both cost and detection probability. Another important consideration is that because eDNA sampling is completely non-invasive, the permitting process could be substantially faster, less labor-intensive, and less costly than more invasive survey methods. Third, eDNA samples can be used as an archive of community composition that extends beyond the current survey goals. Samples collected for one study may be used in later studies for different target species (Dysthe et al. 2018). Finally, it is becoming increasingly clear that preliminary experimentation in the system of interest is crucial for understanding the transport and attenuation dynamics of eDNA particles which can strongly impact monitoring strategies and the interpretation of results. For example, future studies could consider introducing foreign eDNA at a known

concentration to a study system to measure the average eDNA transport distance, to estimate the effects of environmental covariates (e.g., discharge, velocity, depth, substrate, etc.) on transport dynamics, and to facilitate comparisons among studies. As the technology and understanding of eDNA methods continues to improve it is increasingly important that studies such as this are conducted to help guide decision-making processes and increase confidence in eDNA methods for future species monitoring.

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APPENDICES

Appendix A. Supplemental Tables

Table A1. Model selection table of the zero-altered models (with ΔAIC < 2) for predicting counts of juvenile coho salmon in a pool. Models are ranked according to AICc, ΔAIC, and model weight. Covariates that were included (+) or not included (-) in the count and binomial parts of the zero-altered model are identified. The covariates included $\log_{10}$ Basin Area (log(BA)), large woody debris (LWD), the mean of the natural log transformed eDNA concentrations (ln(eDNA)), the residual pool depth (RD) and the offset of the pool area (PA). The covariates included for the count (C.) and binomial (B.) are presented.

Model	C. Log(BA)	C. LWD	C. ln(eDNA)	C. RD	C. PA	B. Log(BA)	B. LWD	B. ln(eDNA)	B. RD	B. PA	AIC$_C$	ΔAIC	Model Weight
1	+	-	-	-	+	-	+	+	+	+	270.7	0	0.10
2	+	-	-	-	+	-	-	+	+	+	271.2	0.43	0.08
3	+	-	-	-	+	-	+	+	-	+	272.2	1.50	0.04
4	+	-	-	-	+	-	-	+	-	+	272.3	1.50	0.04
5	+	-	-	-	+	-	-	+	-	-	272.5	1.72	0.04
6	+	-	-	+	+	-	-	+	+	+	272.6	1.82	0.04
7	+	-	-	-	+	+	-	+	-	-	272.6	1.84	0.04

Appendix B. Cost comparison for eDNA and UVC

The relative cost of implementation is commonly of interest to fisheries professionals when considering the trade-offs of implementing eDNA or conventional surveys. However, such comparisons are challenging given differences in required start-up and operating costs as well as accounting for potential differences in sampling effort (Evans et al. 2017). Despite these challenges, I compared the total costs of labor and materials associated with conducting the Smith River species distribution survey for juvenile coho salmon making several simplifying assumptions. For this comparison, I calculated the approximate cost per reach of surveying with eDNA methods and was provided a cost per reach estimate for the UVC survey by CDFW (S. Ricker and J. Garwood, pers. comm., 2021). These costs include some material startup costs for UVC (e.g., purchase of wetsuits, snorkeling equipment, etc.) and eDNA (e.g., purchase of filters, filter cups, extraction and analysis reagents, etc.); however, not all startup costs were included. Notably, for the eDNA survey, I assumed that all necessary laboratory equipment, space, and species assays were available for use as these are significant monetary investments. Additionally, the costs associated with sample collection (i.e., survey time, travel, lodging, etc.) were not tabulated and assumed to be approximately equivalent between the two approaches as these could vary considerably depending on the setting and number of surveyors. Given these simplifying assumptions, these results should therefore be viewed as conservative estimates of the costs for each survey method.

Based on the budget described below (Table B1), I estimated that the total cost per reach for eDNA was $579, or 18% less than the UVC cost per reach ($707/reach). The reduced labor costs needed for collecting and processing eDNA samples is offset by the greater cost of materials for analyzing eDNA, relative to UVC. 83% of the cost per reach for UVC was for labor (salary + fringe) while eDNA's labor costs was 47% of the eDNA total. These estimates would vary under different assumptions but indicate that the total labor and material costs of eDNA was considerably less than UVC methods.

Budget

UVC:

Personnel. Three surveyors were paid $17/hr to survey one reach per day. The total cost of wages per reach was $510 for a 10-hour day. The fringe rate for CDFW was 16.1% of labor costs, totaling $82.11 per reach for fringe.

Materials. Snorkel materials needed for sampling included wetsuits, dive masks, snorkels, waterproof backpacks, datasheets, etc. The materials cost per reach was estimated to be $18 per reach, under the assumption that materials would need to be replaced after 80 reaches.

Daily expenses: Surveyors were provided a lodging per-diem of $47. Daily fuel costs were estimated at $50 per reach. The total daily expenses would total $97.00 per reach.

eDNA:

Personnel. One graduate student was paid $19.50/hr to collect field samples and process samples in the laboratory. Field sampling times were assumed to be the same as the snorkelers, with 1 reach (10 water samples) completed in 8 hours. Laboratory processing (extraction and analysis) time was estimated as 0.40 hrs per sample, thus the total time to process one reach was 4 hrs. The field labor cost was $156 per reach, and the laboratory labor cost was $78 per reach, with a total labor cost of $234 per reach. A fringe rate of 10.89% was used, according to the Sponsored Programs Foundation at Cal Poly Humboldt, totaling $25.48 per reach.

Materials. The cost of the eDNA filtration materials (e.g., filter cups, filter pads, filter) was $8.53 per sample. Given that 10 samples were collected per reach on average (9 samples + 1 field blank (FB)), the total cost per reach of filtration materials was $85.30 per reach. The cost per reach of the DNA extraction materials (e.g., Quigen DNeasy Blood & Tissue Kit, QIAshredders) was $5.39 per sample, and $53.90 per reach. The cost per reach of eDNA analysis materials (e.g., ddPCR 96-well plates, ddPCR supermix, ddPCR cartridges, etc.) was $4.04 per sample and $40.40 per reach. The total materials cost per reach for filtration, extraction, and analysis was $179.60 per reach. For more information on materials included in each step, see molecular methods.

Daily expenses: The eDNA surveyor was not provided a lodging per-diem but utilized the UVC surveyors' lodging. In the absence of the UVC surveyors this expense would total $47 per reach. Fuel costs were assumed to be identical to the UVC surveyors at $50 per reach. The total daily expenses would total $97.00 per reach

Table B1. Materials, labor, and survey expenses of eDNA and UVC surveys over the 2020-2021 survey period.

Method	Category	Item	Description	Cost per reach (USD)
UVC	Labor	UVC sampling	Total time and cost to survey one reach per day (3 surveyors @ $17/hr; 10hr)	510.00
	Labor	Fringe	Fringe calculated as 16.1% of labor costs	82.11
	Materials	Snorkeling equipment	approx. cost of snorkeling equipment per reach ($1440 for 80 reaches)	18.00
	Travel	Survey costs	$47 camping per diem @ 1 per reach	47.00
	Fuel	Survey costs	$50 per reach	50.00
			TOTAL (UVC)	**707.11**
eDNA	Labor	eDNA field sampling	Cost to survey one reach (1 surveyor @ $19.50/hr; 10hr)	195.00
	Labor	eDNA Laboratory processing	Cost to process (extract and analyze) one reach of samples (1 processor @ $19.50/hr; 0.4 hrs/sample; 10 samples)	78.00
	Labor	Fringe	Fringe calculated as 10.89% of labor costs	29.73
	Materials	eDNA filtration	Cost of filtration materials to survey one average reach ($8.53 per sample; 10 samples per reach)	85.30
	Materials	eDNA extraction	Cost of materials needed to extract all DNA samples from one reach ($5.39 per sample; 10 samples per reach)	53.90
	Materials	eDNA analysis	Cost of eDNA analysis materials for one reach ($4.04 per sample; 10 samples per reach)	40.40
	Travel	Survey costs	$47 camping per diem @ 1 per reach	47.00
	Fuel	Survey costs	$50 per reach	50.00
			TOTAL (eDNA)	**579.33**

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