Comparison of environmental DNA and underwater visual count surveys for detecting juvenile Coho Salmon in small rivers

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Suggested running head: Comparing environmental DNA and underwater visual count surveys

[A] ABSTRACT

Objective

This study compares the probability of detecting juvenile Coho Salmon (*Oncorhynchus kisutch*) using both environmental DNA (eDNA) techniques and underwater visual count (UVC) surveys in northern California rivers. Here, UVC surveys commonly have detection probabilities (p) surpassing 0.90, providing an ideal setting to examine the performance of newer eDNA methods. We also evaluate the potential for using eDNA concentrations to predict the count of Coho Salmon within pool habitats.

Methods

We conducted paired eDNA and UVC surveys in 96 pools across 25 stream reaches within the Smith River basin, California. Method-specific p and the effect of environmental covariates were estimated using multi-scale occupancy modeling. We used generalized linear models to evaluate the relationship of fish counts to eDNA concentrations and habitat covariates.

Results

The eDNA and UVC methods showed a high degree of agreement in detecting the presence of Coho Salmon within a pool (93% agreement) and survey reach (80% agreement). Detection probabilities for eDNA (p_{eDNA}) and for UVC (p_{UVC}) were similar and high at median levels of pool residual depth and contributing basin area (p_{eDNA}=91%, p_{UVC}=89%). Contributing basin area (a proxy for discharge) had a strong, negative effect that was more pronounced for p_{eDNA} than for p_{UVC} (e.g.,in the largest basins, p_{eDNA} = 34% whereas p_{UVC} = 77%). We did not find eDNA concentrations to be a good predictor of Coho Salmon counts in small pools.

Conclusion

This study demonstrates that eDNA methods yielded nearly identical results to UVC surveys in catchments less than 36 km^2 and can provide a highly effective approach for determining the distribution of Coho Salmon. However, additional investigation is required before eDNA could be used to estimate relative abundance in small pools.

Impact Statement. — Environmental DNA was a highly effective survey method for detecting juvenile Coho Salmon in streams, yielding detection probabilities >0.90 under most conditions, but eDNA was not as good of a predictor of the relative abundance in small pools measured in the UVC surveys.

[A] INTRODUCTION

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). Monitoring species' geographic distribution through space and time is critical for the conservation of depleted populations (e.g., McElhany et al. 2000), but tracking the spatial structure of populations 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 they bias estimates of species distributions if not accounted for. To address these challenges, monitoring programs often require extensive survey efforts and may utilize multiple survey

53 methods to maximize detectability for more accurate quantification of population spatial
54 distribution (Nichols et al. 2008).

55 Underwater visual count (UVC) surveys are commonly used to monitor the distribution
56 and abundance of aquatic species (Hankin and Reeves 1988; Thurow 1994). Underwater visual
57 surveys via direct (e.g., snorkeling) or indirect (e.g., camera stations) observation are often used
58 in remote areas due to minimal gear requirements or when environmental conditions (e.g., deep
59 water or high conductivity) limit the effectiveness of other methods such as seining or
60 electrofishing (Thurow 1994; Albanese et al. 2011). The minimally invasive nature of UVC
61 surveys makes them well-adapted for surveys of sensitive or imperiled species such as those
62 listed by state or federal agencies. However, UVC surveys are prone to imperfect detection
63 especially when abundance is low, when species are morphologically similar, or when field
64 observations are limited by water clarity, depth, or habitat complexity (Thurow et al. 2012;
65 Staton et al. 2022; see also Gu and Swihart 2004; MacKenzie et al. 2018).

66 Environmental DNA (eDNA) is a rapidly expanding and promising tool for assessing the
67 distribution and abundance of aquatic species (Thomsen and Willerslev 2015; Rodríguez-
68 Ezpeleta et al. 2021). Several studies suggest that eDNA methods are more rapid, cost-effective,
69 and sensitive than conventional survey methods, particularly when surveying for rare or
70 endangered species (Laramie et al. 2015; Strickland and Roberts 2019; Sutter and Kinziger 2019;
71 Spence et al. 2021; Yu et al. 2021). Compared to conventional monitoring methods, eDNA
72 surveys offer a notable advantage in species detection due to the broad eDNA plumes emitted by
73 aquatic organisms, resulting in nearly double the detection rates in some studies (Schmelzle and
74 Kinziger 2016; McColl-Gausden et al. 2021; Dougherty et al. 2016; Valdivia-Carrillo et al.

2021). Successful detection of a target species with eDNA methods is influenced by the quantity, distribution, and attrition of eDNA in a system, which in turn depend on the characteristics of the target species (e.g., behavior, abundance, distribution, size, DNA shedding rate, and the form of eDNA produced; Turner et al. 2014; Yates et al. 2019; Castañeda et al. 2020, Andruszkiewicz Allan et al. 2021), and conditions that govern the movement and attrition of eDNA in the system (e.g., discharge, velocity, substrate, temperature, ph, salinity; Baldigo et al. 2017; Mize et al. 2019; Harrison et al. 2019; Spence et al. 2021; Wood et al. 2021; Yates et al. 2021). These factors can influence the efficacy of eDNA methods to a degree that requires eDNA monitoring strategies to be tailored to the location and the species of interest (Goldberg et al. 2016; Yates et al. 2021).

Occupancy modeling frameworks have been successfully applied to eDNA datasets to account for the imperfect detection of eDNA in water samples and for the influence of environmental factors on occupancy and detectability (e.g., Schmelzle and Kinziger 2016; Sutter and Kinziger 2019; Smith and Goldberg 2020; Martel et al. 2021). The hierarchical nature of eDNA surveys yields data that is highly suited to analysis using a multi-scale occupancy framework to estimate occupancy patterns at multiple spatial scales while accounting for environmental covariates and differences in survey method (Nichols et al. 2008; Dorazio and Erickson 2017; MacKenzie et al. 2018). For example, multi-scale occupancy models can be used to estimate and compare the detection probabilities of concurrently applied survey methods, providing critical information for survey design and method-specific effectiveness (Nichols et al. 2008).

Study designs that utilize eDNA methods in conjunction with other conventional survey methods to compare results are common in the literature, but the formal quantification and comparison of method-specific detection probabilities is limited, particularly for river systems (e.g., Castañeda et al. 2020). Fediajevaite et al. (2021) found that only 18 of 535 studies using eDNA methods (3%) provided a quantitative comparison of eDNA to conventional survey methods via estimation of method-specific detection probabilities. 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, California. Coho salmon inhabiting the Smith River are part of the Southern Oregon/Northern California Coast Evolutionarily Significant Unit and are currently listed as threatened under the US Endangered Species Act. The California Department of Fish and Wildlife (CDFW) uses UVC methods (i.e., snorkeling) to determine the spatial distribution of juvenile Coho Salmon in the Smith River each summer (Walkley and Garwood 2017), and we integrated eDNA collections into CDFW's pre-existing survey protocols over two survey seasons (2020-2021). The detection capabilities shown in the CDFW snorkeling surveys are very high (>0.9; Walkley and Garwood 2017) and create an optimal environment for precise benchmarking of eDNA methods. This study had two primary objectives: (1) to compare the ability of eDNA and UVC surveys to detect Coho Salmon and assess the influence of

environmental covariates on detection probabilities and (2) to evaluate the potential for using eDNA concentrations and habitat covariates to predict the count of Coho Salmon within pools.

[A] METHODS

[B] *Study site*

The Smith River drains a watershed spanning 1,862 km^2 of northern California and southern Oregon (Figure 1). Nearly all of the basin (98%) is within the Klamath and Siskiyou Mountains. The Smith River is the largest free-flowing coastal river in California; there are no dams and anadromous fishes have access to the entire basin (Garwood and Larson 2014; Walkley and Garwood 2017).

[B] *Field methods*

During July and August of 2020 and 2021, eDNA sampling was conducted in conjunction with the annual UVC survey of the Smith River basin executed as part of the CDFW Coastal Salmonid Monitoring Program (Adams et al. 2011; Garwood and Ricker 2016; Walkley and Garwood 2017). For the UVC survey, sampling units of approximately 1-3 km in length (hereafter reaches) were selected using a generalized random tessellation stratified algorithm out of 166 total sampling units that represent all juvenile salmonid-rearing habitat in the Smith River basin during summer baseflow conditions (Figure 1; Stevens and Olson 2004; Garwood and Larson 2014). Garwood and Larson (2014) identified potential salmonid rearing habitat using models of physical stream attributes (i.e., maximum gradient and minimum discharge thresholds), information on salmonid migration barriers, and known salmonid distributions.

Model predictions were split into distinct reaches at natural or artificial breaks (i.e., tributary confluences, bridges), with terminal ends at barriers to adult migration (For additional information please see Garwood and Larson 2014). The eDNA samples were collected in all non-mainstem UVC survey reaches in 2020 and in a subset of the non-mainstem UVC reaches in 2021, yielding a total of 26 reaches (19 in 2020 and 7 in 2021). One reach was surveyed in both years but was treated as two independent samples because Coho Salmon typically spend only one summer rearing in streams (Brown et al. 1994). In 2021, extra eDNA sampling occurred selectively in UVC reaches with historically higher numbers of observed juvenile Coho Salmon (Walkley and Garwood 2017); this was done to increase the sample size of occupied reaches and to increase the range of observed fish counts in the data set because few reaches were found to be occupied in 2020. Any reaches from 2021 where eDNA samples were not collected were excluded from our analysis.

Systematic sampling was used to select UVC survey pools within a survey reach. Only pools that met minimum habitat criteria of depth, size, temperature, and visibility, which varied according to the mean annual discharge of reach, were included in the UVC survey (for further information see Garwood and Ricker (2016). A coin flip decided which of the first two pools was the starting point of the UVC survey, after which every alternate pool was surveyed. In the first sampled pool, two divers conducted independent census counts of juvenile Coho Salmon (referred to as a “double-pass” pool). Every other upstream pool was surveyed systematically, with the next three surveyed pools only getting a single pass by one diver; this sequence (i.e., 2-0-1-0-1-0-1-0) was repeated for the remainder of the reach. For each double-pass pool, the second diver waited approximately five minutes after the first diver’s pass for disturbed sediment in the pool to settle. When surveying a pool, divers proceeded upstream, examined the entire

160 width of the pool, and recorded the number of juvenile Coho Salmon present. Divers also
161 recorded the number of large woody debris (LWD; >30 cm in diameter), the residual pool depth
162 (RD; the difference in height between the deepest point in a pool and the downstream riffle crest;
163 Lisle 1987), the total pool length, and the average pool width. Additionally, the contributing
164 basin area (BA) to each survey reach was used as a proxy for river discharge as the two measures
165 were assumed to scale geometrically (Galster 2007). The BA values were obtained using the
166 StreamStats application (U.S Geological Survey 2016) and assumed to be constant for a given
167 survey reach. On average, reaches were 2.1 km in length and there were four double-pass pools
168 per survey reach (max of 11, min of 1). Within a reach, individual survey pools were on average
169 17 m long, 6 m wide, 157 m apart, and the double-pass pools were 536 m apart. LWD counts
170 ranged from 0-11 structures per pool, RD ranged from 1-320 cm per pool, and BA ranged from
171 0.26-155 km^2 per reach (Figure S1).

172 Water samples for eDNA analysis were collected at all double-pass pools with a few
173 exceptions. When reach lengths were greater than 2 km or when many (>30) pools were
174 expected, water samples were collected at every other double-pass survey pool. All water
175 samples were collected before divers entered the water, by personnel dedicated to water sample
176 collection (i.e., not divers). At each pool selected for eDNA sampling, three 1-liter water samples
177 were collected using single-use Whirl-Pak bags (Nasco). Water samples were obtained by
178 drawing a Whirl-Pak bag along the water's surface in the thalweg at the downstream end of a
179 pool. All samples were filtered in the field immediately after collection, across 0.45-micron
180 cellulose nitrate filters (Cytvia), held in filter funnels (Thermo Scientific™ Nalgene™ Single-
181 Use Analytical Filter Funnels). Filter funnels were held in a filtration manifold that allowed up to
182 four samples to be filtered simultaneously using a manual vacuum pump. Filter support pads

183 (MilliporeSigma™) were used to ensure equal filtration across the surface of the filter. A field
184 blank was collected at least once per survey day by filtering 1 liter of store-bought drinking
185 water. Field blanks were processed the same as the other samples and served as comprehensive
186 contamination controls. After filtration, filters were placed into 2 ml microcentrifuge tubes
187 (Eppendorf) containing 360 µL of cell lysis buffer (QIAGEN, buffer ATL). Samples remained
188 unfrozen for a maximum of three days post-filtration due to the remote nature of some survey
189 locations but were stored at -20°C upon returning from the field.

190 [B]*Molecular methods*.— The DNA was extracted directly from filters using the QIAGEN
191 DNeasy Blood and Tissue Kits following the manufacturer's instructions with three exceptions:
192 1) we used 360 µl of buffer ATL and 40 µl proteinase K (Schmelzle and Kinziger 2016), 2)
193 QIAshredders (QIAGEN) were used to ensure lysate homogenization, and 3) during the final
194 elution step, 100 µl of elution buffer was used to increase the final DNA concentration of the
195 elution. All extractions were completed within three months of field collection and extracted
196 DNA was stored at -20 °C.

197 The concentration of eDNA in a sample was estimated using droplet digital PCR
198 (ddPCR) with the Bio-Rad QX200 Droplet Digital PCR System. Each ddPCR reaction was run
199 in duplex using assays for Coho Salmon (developed by Pilliod and Laramie (2016), modified by
200 Spence et al. 2021) and Chinook salmon (*Oncorhynchus tshawytscha;* [Unpublished, U.S. Forest
201 Service National Genomics Center for Wildlife and Fish Conservation at the Rocky Mountain
202 Research Station, Missoula, Montana]). Assay specificity and sensitivity were verified by testing
203 against co-occurring species: Coho Salmon, Chinook salmon, steelhead *Oncorhynchus mykiss,*
204 and Coastal Cutthroat Trout *Oncorhynchus clarkii clarkii*. Fluorescence plots indicated signal

interference in the Chinook salmon channel when the Coho Salmon assay was present, leading to the exclusion of the Chinook data from any subsequent analysis or 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, 0.2 µl of 300 mM dithiothreitol, 15 µl of DNA template to maximize the probability of target DNA presence in the analyte (Rees et al. 2014; Doi et al. 2015), and DNA-free 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 were placed into individual wells of a Bio-Rad DG8 cartridge and placed into the Bio-Rad QX-200 droplet generator which partitions the reaction mix into as many as 20,000 droplets for PCR amplification. Partitioned samples were transferred to 96 well ddPCR plates, sealed with a PX1 PCR plate sealer and placed in an MJ Research PTC-100 Thermal Cycler. Thermocycling conditions were 10-minutes at 95°C followed by 40 cycles of 30-seconds at 94°C and 60-seconds at 60°C, and concluded with 10-minutes at 98°C, and holding at 4°C. The temperature ramp rate was set to 2°C between all steps. The number of positive and negative droplets in each reaction were determined using a QX200 droplet reader. Concentrations are reported as the Poisson-corrected copies per reaction as estimated by the Bio-Rad QX Manager Software Version 1.2. Each ddPCR plate run contained at least one positive control (genomic DNA extracted from the tissue of the target species) and at least one negative control (containing all reagents except DNA template, which was replaced with DNA-free water). 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 results of the second analysis were used.

All DNA extractions and ddPCR setups were conducted in a dedicated low-copy eDNA laboratory. 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 entered the separate dedicated lab designated for any high concentrations of DNA (e.g., from running PCR reactions).

Limits of detection (LOD) and quantification (LOQ) were determined using serially diluted genomic DNA extracted from fin clips using Qiagen DNeasy Blood and Tissue Kits. The LOD, defined as the lowest concentration of DNA resulting in at least 95% positive detections, and LOQ, defined as the lowest concentration with a coefficient of variation below 35%, were determined using curve fitting methods. Positive detections were indicated by samples for which measured concentrations of Coho Salmon eDNA exceeded the LOD (7 DNA copies per reaction). Estimates of eDNA concentration that exceeded the LOQ (47 DNA copies per reaction) were considered to be accurate measures.

[B] *Occupancy analysis*

Multi-scale occupancy models were used to estimate and compare the method-specific detection probabilities for eDNA and UVC. Models were fitted in a maximum-likelihood framework using the software PRESENCE (version 2.13.10; Hines 2006). The occupancy model included three parameters at different hierarchical levels: Psi (Ψ) is the probability of species occurrence in a river reach; theta (θ_t) describes the probability of the species occurrence in any given pool *t* of the larger survey reach which is conditional on the species being present within

247 the reach; and $p_{m,t}$ describes the probability of the species being detected by survey method *m* in
248 pool *t* of the survey reach, conditional upon the species being present in both the reach and the
249 pool. This analysis only included data from the double-pass survey pools with both eDNA and
250 UVC observations. A detection history for a survey pool would have five digits, with the first
251 two numbers representing detections from each of the two dive passes and the last three numbers
252 representing detections from the three eDNA samples. For example, a detection history of 11101
253 in a survey pool would indicate that Coho Salmon were detected in the pool by both divers and
254 in eDNA water samples one and three, and a detection history of 00000 would indicate that
255 neither method detected Coho Salmon. Note that this parameterization differs from some other
256 applications of hierarchical modeling of eDNA in which p for eDNA is defined as the
257 probability of detecting Coho Salmon DNA in a replicate quantitative PCR (qPCR) run within a
258 single water sample and θ is the probability that the water sample contains Coho Salmon DNA
259 (Schmidt et al. 2013; Schmelzle and Kinziger 2016; Dorazio and Erickson 2017; Spence et al.
260 2021).

261 The analysis was structured to assess the influence of several covariates that potentially
262 affect detection of Coho Salmon by either or both survey methods. We hypothesized that UVC
263 detection probability would be reduced by increasing RD and LWD due to difficulties in
264 observing individuals in deeper water or with visual obstructions (e.g., Thurow et al. 2006;
265 Staton et al. 2022) and that eDNA detection probability would be reduced by increasing BA due
266 to the dilution of eDNA particles (Baldigo et al. 2017). For this analysis, both RD and BA were
267 $\log_{10}$ transformed. Finally, a covariate for year was included to account for possible differences
268 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 the target species. Count was also considered as a covariate but was not included in the final analysis, due to model conversion issues. Note that we did not assess the influence of covariates on Ψ or θ because our focus was to compare detection probabilities rather than determine species occupancy patterns and because site selection for eDNA methods was not random in 2021.

Model selection was done using Akaike's information criterion (AIC) with a suite of 23 models defined by nearly all possible combinations of the covariates (method, BA, RD, LWD, year). Aside from a null model, where the detection probabilities were constant, 'method' was included in every model fit. Covariates were included individually and with interactions by method (with the exception of a year-method interaction). Occupancy models were ranked using AIC, AIC differences (ΔAIC), and AIC weights (Burnam and Anderson 2002). All models within a ΔAIC of 2 were considered as competing models supported by the data.

For the best model, we generated response plots to assess the influence of each covariate on detection probabilities. Predictions were made over the observed range of values for a covariate while all other covariates were held at their median values. A Monte Carlo approach was used to approximate the standard error (SE) of 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 randomly drawn coefficients was used to generate a response curve for each covariate (while holding the other covariates at their medians). The SE for the response plots was approximated using the distribution of 1000 Monte Carlo predictions generated for each covariate value.

The cumulative probability of detection (p^*) was calculated as $p^* = 1 - (1-p_t)^n$, where n is the number of replicate water samples taken from a pool that contained Coho Salmon DNA. Cumulative detection probabilities were calculated for the highest, median, and lowest estimates of p_t for the sampled pools based on the observed covariates and the best occupancy model. Plots of p^* as a function of sample size were analyzed to estimate the sampling effort required to detect Coho Salmon DNA with 95% cumulative probability under the observed values of covariates (McArdle 1990).

[B]*Concentration-count analysis*

To assess the potential for estimating abundance of Coho Salmon in the absence of visual counts, we fit a generalized linear model (GLM) that related the average of the two counts from the double-pass survey pools (rounded to the nearest integer), to the natural log (ln) of the average of the three eDNA concentrations [ln(copies/reaction)] and the three habitat covariates: LWD [count], RD [log10(cm)] and BA [log10(km2)]. To account for variation in pool size, an offset of the natural log of pool area was also included, where pool area was calculated using the product of the maximum pool length and the average pool width. This analysis excluded data from pools in which no fish were observed. Initial analysis of models including all variables indicated that a zero-truncated, generalized Poisson error distribution yielded superior fits compared to zero-truncated Poisson or Negative Binomial distributions based on AICc and residual variance diagnostics (Zuur et al. 2009). A total of 32 models with all possible combinations of the covariates and the area offset were fit and compared by AICc using packages *glmmTMB* (Brooks et al. 2017) and *MuMIn* (Bartoń 2020). Model diagnostics (e.g., residual variance, overdispersion, data distribution) were assessed using package *performance*

(Lüdecke et al. 2021). The best model was used to estimate the effects of the covariates on mean fish count while holding all other covariates at their median values.

[A] RESULTS

[B] Survey results

A total of 114 double-pass pools and 318 single-pass pools distributed among 25 reaches were surveyed in 2020 and 2021. Of the double-pass pools, 96 were surveyed with both eDNA and UVC methods. Diver counts of juvenile Coho Salmon ranged from 0-210 fish per pool (mean = 32; Figure S1). The difference between the two independent dive counts ranged from 0 to 81 (mean = 4), but there was 100% agreement between divers regarding whether a pool was occupied or not. None of the field blanks or negative PCR controls tested positive for Coho Salmon eDNA. All of the positive controls were positive for Coho Salmon eDNA.

Survey methods yielded identical results with respect to the detection and non-detection of Coho Salmon in 93% (89 of 96) of pools in which both surveys were conducted (27 presences; 62 absences; Table 1), indicating strong agreement between the two methods (Pearson's Chi-squared test; χ^2=63.00, p<<0.001). Similarly, the two methods agreed at 80% (20 of 25) of survey reaches (7 presences; 13 absences; Table 1) and the relationship was statistically significant (Pearson's Chi-squared test with simulated p-value based on 2000 replicates; χ^2=7.77, p=0.013). Coho salmon eDNA was detected in three reaches where none were observed, and Coho Salmon were observed in three (different) reaches where no eDNA was detected.

[B] Occupancy modeling

Five of the 23 occupancy models examined had a ΔAIC less than two (Table 2) and were far more supported than the null model with no covariates for *p* (ΔAIC of 19.96). The top model (AIC Weight = 0.25) included survey method, RD, BA, and a method-BA interaction as covariates for detection probability. Survey method, RD, and BA were included in all five models while all other covariates were less consistent (Table 2). For plotting the response, we focused on the model with the highest model weight for simplicity.

At median values of RD and BA, the estimated detection probability of eDNA (p_{eDNA}=0.91; 95% CI: 0.80 - 0.96), was very similar to the estimated detection probability of UVC (p_{UVC}=0.89; 95% CI: 0.79 - 0.94; Figure 2A). Estimated detection probabilities for both methods increased with RD; p_{UVC} ranged from 0.61 – 0.94 and p_{eDNA} from 0.68 – 0.96 (Figure 2B). Although the large standard errors of predictions at low RD values were strongly influenced by a single data point, the predicted response was unaffected by exclusion of this observation. Increasing BA had a strong negative effect on both methods, but the effect was more pronounced for eDNA; as $\log_{10}$(BA) increased beyond 1.55 (i.e., BA > 36 km^2), p_{eDNA} declined rapidly from 0.99 reaching 0.34 when BA > 100 km^2, while p_{UVC} declined more gradually from 0.98 to 0.77 (Figure 2B). Across the surveyed pools, the range of the site-specific estimates of detection probability for p_{eDNA} values (0.99 to 0.13) varied more widely than p_{UVC} (0.97 to 0.42).

Under our sampling protocol, triplicate water samples were sufficient to achieve cumulative detection probabilities >95% at nearly all (98%) of the surveyed pools. Given the presence of Coho Salmon DNA in a pool with median values of RD and BA, it would be detected with high probability (p*= 0.91, 95% CI: 0.79 - 0.95) in a single water sample, and with >95% probability in two samples (Figure 3). Under conditions that strongly suppress the

356 detection of eDNA, particularly sites with BA values greater than 100 km^2, twenty or more
357 samples would be required to achieve >95% cumulative probability of detection.

358 [B] Concentration-count analysis

359 GLM models explained observed fish counts in pools well, but eDNA was not an
360 important predictor in the best model. Competing GLMs (with ΔAIC<5) consistently included
361 the pool-area offset and basin area as covariates, whereas LWD, eDNA, and RD only occurred in
362 models with ΔAICc > 2 (Table 3). The best model had a model weight of 47% (Table 3) and had
363 a strong correlation between observed and predicted fish counts in pools (R^2=0.83). Fish count
364 was predominantly driven by a positive relationship with pool area that accounted for larger
365 pools being able to hold more fish at a given fish density (all else being equal), but there was also
366 strong evidence of lower counts in reaches with larger basin areas (Figure 4), which is consistent
367 with juvenile coho habitat preferences for small streams (Brown et al. 1994).

368 [A] DISCUSSION

369 In this study, we demonstrate that eDNA methods are comparable to UVC surveys
370 known to have exceptionally high detection probabilities for juvenile Coho Salmon in our
371 research system. Our findings suggest that these eDNA methods have the potential to
372 substantially enhance sensitivity and could be a suitable alternative to UVC surveys in settings
373 where species detection rates are much less than 1, which is common in most field surveys
374 (Fediajevaite et al. 2021). We found that p_{eDNA} was effectively equivalent to p_{UVC} in reaches with
375 contributing basins < 36 km^2, but p_{eDNA} declined sharply in reaches with larger basin areas and
376 with presumably higher discharge. Two of the three instances where eDNA methods failed to

377 detect Coho Salmon where UVC were successful occurred in the reaches with the highest BA's
378 (i.e., 115 and 155 km^2). Our results also corroborate other studies, demonstrating that eDNA is a
379 highly effective method for detecting rare species (McKelvey et al. 2016; Rice et al. 2018;
380 Strickland and Roberts 2019; Sutter and Kinziger 2019, Fediajevaite et al. 2021, Spence et al.
381 2021). However, additional investigations are necessary to determine whether eDNA methods
382 could provide a reliable tool for predicting juvenile Coho Salmon abundance in small pools.

383 This study demonstrated that eDNA methods have the capability to yield similar
384 estimates of Coho Salmon spatial distribution with less overall sampling effort. Specifically,
385 UVC and eDNA methods agreed on the presence or absence of Coho Salmon in 80% of
386 surveyed reaches, despite differences in sampling intensity (432 pool surveys with UVC; 96 with
387 eDNA). These results align with earlier investigations conducted by Evans et al. (2017) and Yu
388 et al. (2021) that showed eDNA methods require less sampling effort than some conventional
389 survey methods. Studies have also shown eDNA methods can be more cost effective than
390 conventional methods, although we did not conduct a detailed cost comparison in this study
391 (Evans et al. 2017; Fediajevaite et al. 2021; Yu et al. 2021).

392 The best occupancy model from our analysis indicates that both methods had a high
393 probability of detecting Coho Salmon in a pool (~90% in typical settings), but those detection
394 probabilities were influenced strongly by BA and weakly by RD. In general, the eDNA detection
395 probabilities were high and greater than that of UVC until the median BA (18 km^2), at which
396 point eDNA detection probabilities began to decline more rapidly than those of UVC. These
397 results are consistent with the hypothesis that increasing discharge, for which BA is a proxy,
398 would decrease the probability of capturing and detecting rare organismal DNA due to the

dilution of particles (Levi et al. 2019, Pochardt et al. 2020). However, the effect of discharge on observed eDNA concentrations can be complex and dependent upon numerous factors that may vary across time and space (e.g., eDNA plume dynamics, increased turbidity and presence of inhibitors, eDNA particle settling and resuspension dynamics; Jane et al. 2015, Wilcox et al. 2016; Matter et al. 2018, Wood et al. 2021, Van Driessche et al. 2023). In this study, the negative BA effect for eDNA may have been more substantial because the eDNA sampling effort per pool was fixed (three samples per pool), whereas the sampling effort for UVC (in terms of area surveyed) was commensurate with pool size. The slight decrease in method-specific detection probabilities at lower values of RD may have resulted from faster water velocities in survey pools with low values of RD which could have hindered UVC divers and diluted available eDNA as these sites were more similar to runs or riffles; for example, Wood et al. (2021) found that the amount of available eDNA in the midstream water column was generally lower and more variable in areas with high velocity than where velocities were low.

In river systems, eDNA is carried from the source organism and can be detected downstream if eDNA concentrations remain sufficiently high (Goldberg et al. 2016). Although this eDNA transport can enhance the detectability of a target species throughout an extended spatial area, it undermines the spatial independence of samples assumed within occupancy models (MacKenzie et al. 2018). In the present study, however, the spacing between pools surveyed for eDNA consistently exceeded scales of eDNA transport reported for streams of similar size (survey spacing: 536 m; 95% C.I.: 438 - 635 m, detection ranges of ~200 m [Spence et al. 2021; see also Jo and Yamanaka 2022]), supporting our modeling assumption of independence across sampling locations. It is possible that some eDNA detections could have resulted from fish that were upstream of an unoccupied pool and thus not available for detection

422 via UVC. However, the strong method-specific agreement in pool-level detections suggests this
423 was not a common occurrence nor a substantial source of bias in the occupancy models. Of the
424 few cases where eDNA detected Coho Salmon and UVC did not, fish were not typically
425 observed by divers in the single-pass pools immediately upstream of the eDNA sample pool; in
426 the singular case where this happened, Coho Salmon were detected a substantial distance
427 upstream (300 m) in low numbers (n=3).

428 Although our capacity to use eDNA in water samples to estimate abundance is still
429 developing, a growing body of research demonstrates a positive correlation between eDNA
430 concentration and biomass or abundance (Rourke et al. 2022, Takahara et al. 2012, Schmelzle
431 and Kinziger 2016, Tillotson et al. 2018, Capo et al. 2020, Shelton et al. 2022). In the present
432 study, however, we found that eDNA concentration was not a good predictor of the average
433 count of Coho Salmon in a pool. We suspect that the limited variability in abundance and
434 biomass of juvenile Coho Salmon across sampling units (2-210 individuals per pool [2-orders of
435 magnitude]) contributed to our inability to resolve such a relationship. Studies that have
436 identified strong relationships between eDNA concentration and abundance indices have done so
437 when differences in abundance or biomass (ranging over 3 to 6 orders of magnitude) varied
438 substantially more than in our system (Tillotson et al. 2018; Yates et al. 2019; Pochardt et al.
439 2020; Sepulveda et al. 2021; Shelton et al. 2022).

440 This study and others suggest that eDNA surveys could be a suitable alternative or
441 complement to UVC surveys, but more work is needed to develop robust and optimal sampling
442 designs. Our protocol of collecting triplicate 1-liter water samples was sufficient to achieve a
443 95% probability of detecting Coho Salmon DNA in a pool (if present) in basins up to 70 km^2, but

more sampling effort would be required to achieve similar confidence levels for detection of eDNA in sites with larger contributing basins. Instead of filtering substantially larger volumes of water, which can be logistically difficult in some systems (e.g., filter clogging, increased presence of inhibitors; Capo et al. 2020), future eDNA monitoring efforts should consider increasing the number of water samples commensurate with basin size to maintain high cumulative detection probabilities, or they could limit eDNA surveys to areas with acceptably high performance given sampling constraints. Monitoring programs that use eDNA also need to evaluate appropriate levels of spatial sampling effort (e.g., number of pools sampled within a reach) to achieve desired objectives, and this will depend on the pool-level occupancy rate and detection probability for the target species (see equations in Spence et al. 2021). Based on pool-level occupancy rates of juvenile Coho Salmon in the Smith River (0.47 ± 0.02 SE; Walkley and Garwood 2017) and our median estimated eDNA detection probabilities sampling five pools per reach (using three water samples and 1 ddPCR replicate) was sufficient to yield >95% cumulative probabilities of encountering and detecting Coho Salmon at the reach scale. In other California streams, Spence et al. (2021) found that sampling two locations per survey reach (using 3 water samples and 1-2 PCR replicates) resulted in an overall detection probability of 0.74 - 0.99, depending on Coho Salmon densities. Overall, eDNA can be an effective tool for detecting and monitoring fishes in rivers and streams but monitoring programs using this method should be designed and optimized to achieve desired objectives given case-specific detection and occupancy rates, environmental conditions, and sampling constraints.

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TABLES

TABLE 1. The percentage (and number) 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 an additional 318 pools where only UVC observations occurred, which reflects the current survey protocol.

eDNA Detection	UVC Detection	Pools	Reaches
+	+	28% (27)	28% (7)
-	-	65% (62)	52% (13)
+	-	5% (5)	8% (2)
-	+	2% (2)	12% (3)

TABLE 2. Top five occupancy models (with ΔAICc < 2) for juvenile Coho Salmon from the multi-method occupancy analysis. Covariates that were included (+) in each model are identified. Detection probability at the pool-level was modeled as a function of survey method (m), count of large woody debris (LWD), the $\log_{10}$ of residual pool depth (RD), the $\log_{10}$ of the contributing basin area (BA), Year (Yr), and interactions between the habitat covariates and method (e.g., BA * m). K represents the number of estimated parameters in the model. Differences in AICc values relative to the top-ranked model (ΔAICc) and model weights (W) are provided for all models.

Model Rank	m	LWD	RD	BA	Yr	LWD*m	RD*m	BA*m	K	ΔAICc	W
1	+		+	+				+	7	0	0.25
2	+		+	+					6	1.48	0.12
3	+	+	+	+		+	+	+	10	1.84	0.10
4	+		+	+			+	+	8	1.89	0.10
5	+		+	+	+			+	8	1.95	0.10

Table 3. Model selection table of generalized linear models (with ΔAICc < 5) for predicting non-zero counts of juvenile Coho Salmon in a pool. Models are ranked according to AICc, ΔAICc, and model weight. Covariates that were included (+) in each model are identified. Covariates

included log10 of basin area (BA), large woody debris (LWD), the mean of the natural log transformed eDNA concentrations (eDNA [copies/reaction]), the residual pool depth (RD), and the natural log of pool area (PA) as an offset.

Model	BA	LWD	eDNA	RD	PA	df	AICc	ΔAICc	W
1	+				+	3	253.66	0.00	0.474
2	+	+			+	4	256.14	2.48	0.137
3	+		+		+	4	256.30	2.64	0.127
4	+			+	+	4	256.36	2.70	0.123

FIGURE CAPTIONS

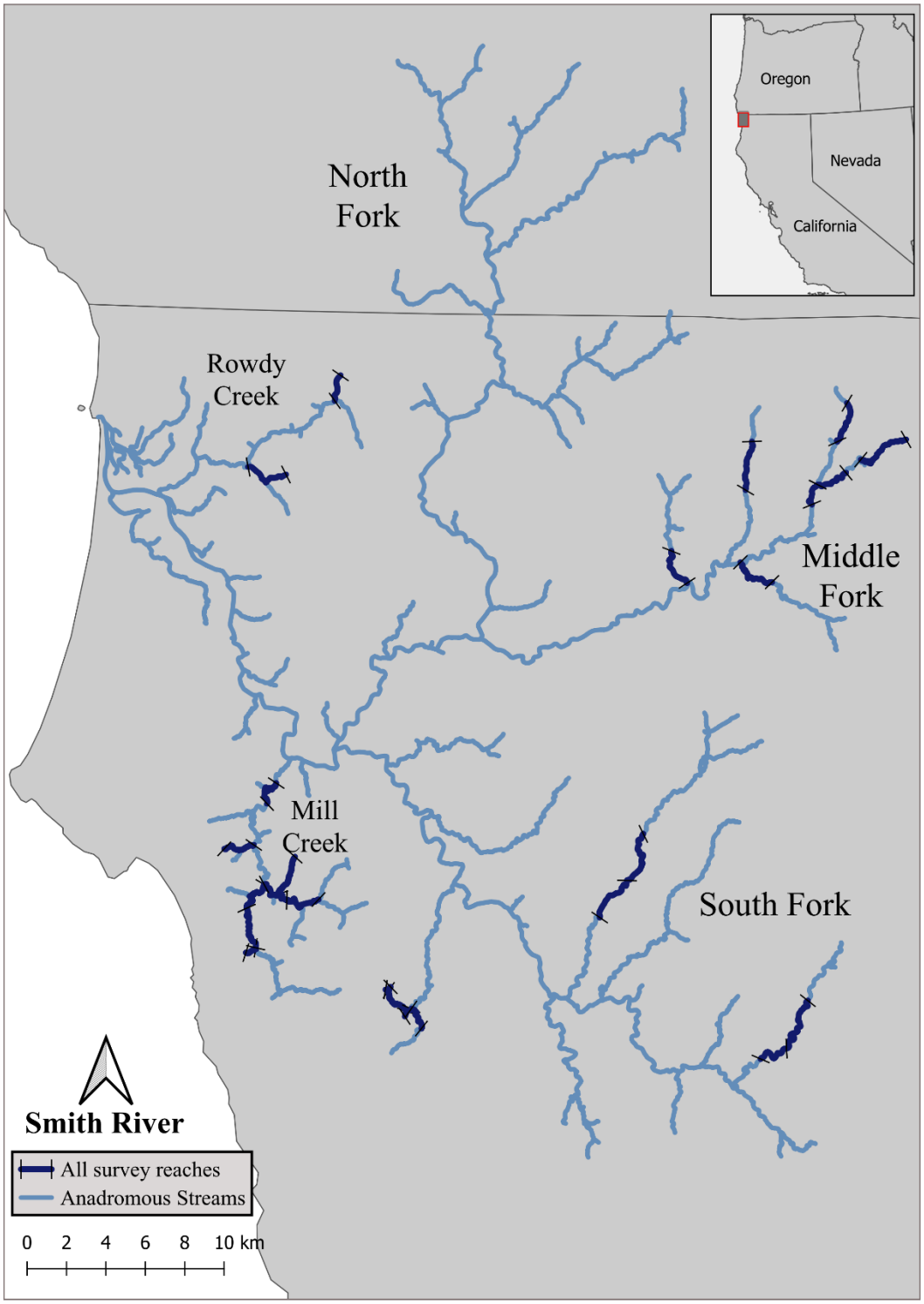

FIGURE 1. The anadromous rearing habitat (light blue lines) of the Smith River (California, USA) and the locations of the 25 surveyed stream reaches (dark blue bolded lines) to compare the ability of eDNA and UVC surveys to detect Coho Salmon.

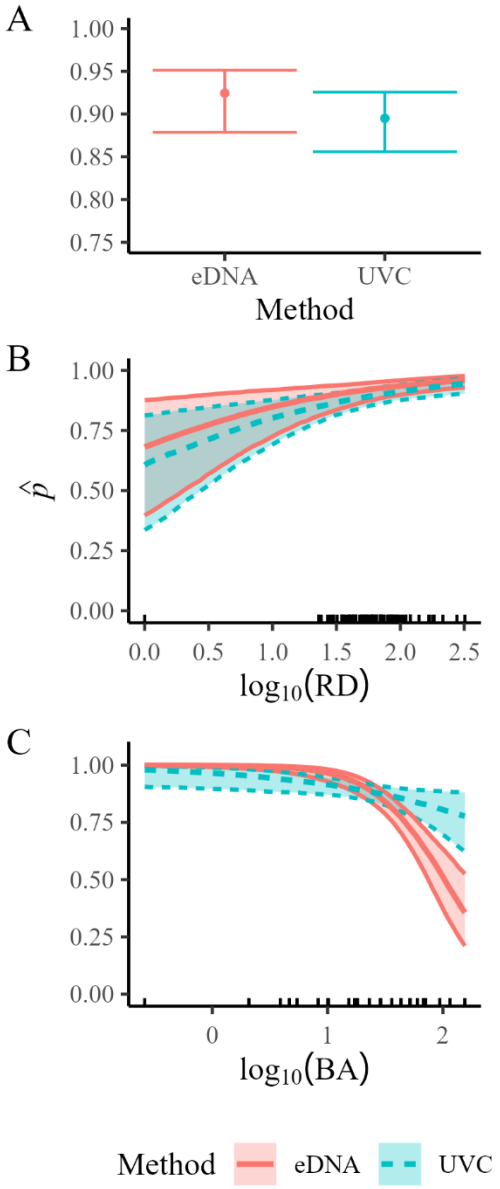

FIGURE 2. Predicted effects of **(A)** survey method, **(B)** $\log_{10}$ residual pool depth (cm;RD), and **(C)** $\log_{10}$ of basin area (km^2; BA) on the detection probabilities (p) for eDNA (thick, solid, red line) and UVC (thick, dashed blue line) with the associated standard error (thin lines of the same color and type). Effect sizes were calculated over the observed range of values for the covariate, shown as ticks (i.e., rug), while all other covariates were held at their median values. Observed values for **(B)** are the unique RD for each pool, while **(C)** is the BA at the reach level and was applied to all pools in the reach.

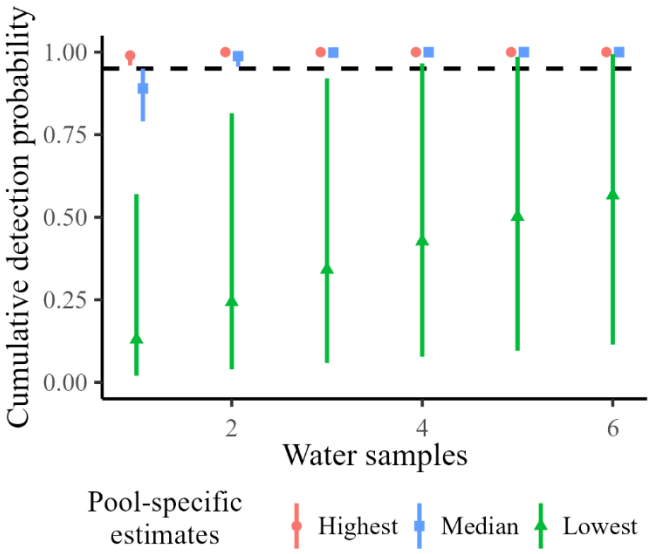

FIGURE 3. The cumulative detection probability as a function of the number of replicate water samples, calculated using the highest (p = 0.99), median (p = 0.89), and lowest (p = 0.13) estimated pool-specific detection probabilities. The vertical bars represent the 95% confidence interval. The horizontal dashed line represents the 95% cumulative detection probability.

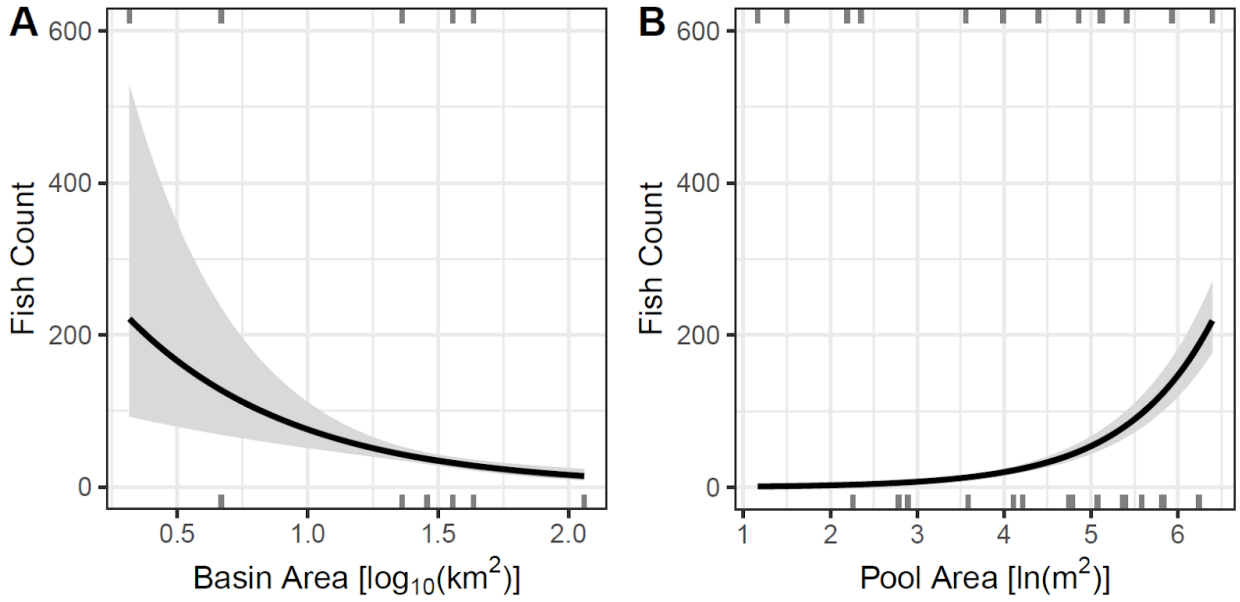

FIGURE 4. Predicted effects of (**A**) basin area and (**B**) an offset for pool area on the predicted mean count of Coho Salmon in a survey pool based on the top generalized linear model. Solid lines are the predicted effect of the variable (with 95% confidence interval) when all other model covariates are held at their median value. Ticks (i.e., rug) on horizontal axes denote location of positive and negative partial residuals.

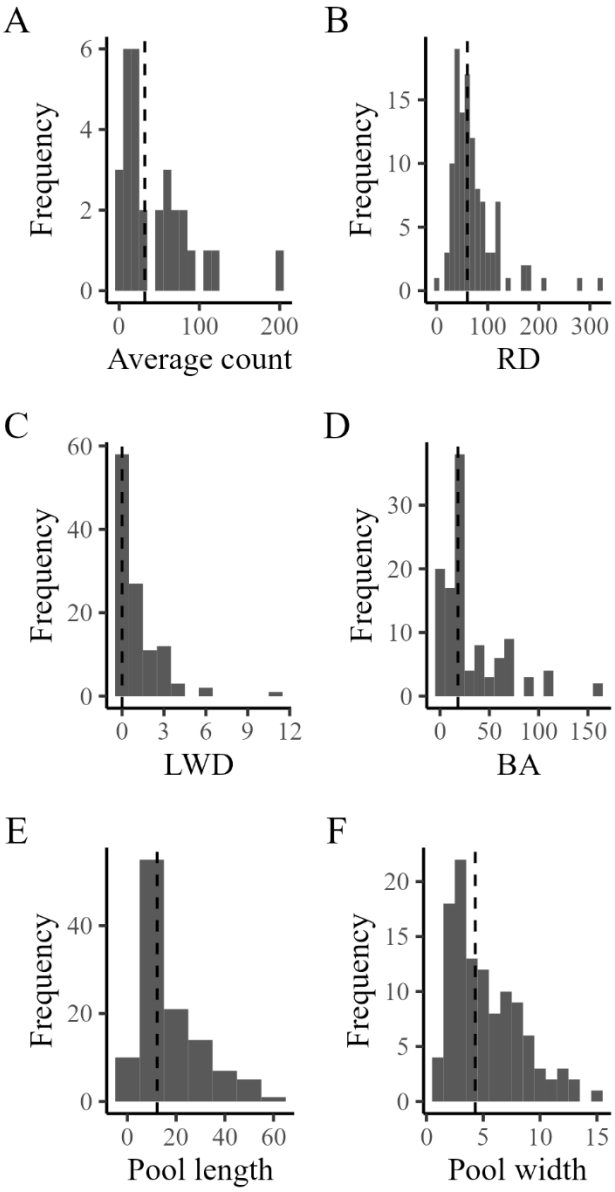

FIGURE S1. Frequency distributions of **(A)** the average count per occupied reaches of coho salmon, **(B)** the Residual Depth (RD in cm), **(C)** Large Woody Debris (LWD), **(D)** Basin Areas (BA in km^2), (**E**) Pool length (m), and (**F**) Pool width (m) observed in the 2020-2021 survey seasons. Median values are shown (vertical dashed lines).