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ARTICLE

It's complicated . . . environmental DNA as a predictor of trout and char abundance in streams

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Abstract: The potential to provide inferences about fish abundance from environmental (e)DNA samples has generated great interest. However, the accuracy of these abundance estimates is often low and variable across species and space. A plausible refinement is the use of common aquatic habitat monitoring data to account for attributes that influence eDNA dynamics. We therefore evaluated the relationships between eDNA concentration and abundance of bull trout (*Salvelinus confluentus*), westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and rainbow trout (*Oncorhynchus mykiss*) at 42 stream sites in the Intermountain West (USA and Canada) and tested whether accounting for site-specific habitat attributes improved the accuracy of fish abundance estimates. eDNA concentrations were positively associated with fish abundance, but these relationships varied by species and site, and there was still considerable variation unaccounted for. Random site-level differences explained much of this variation, but specific habitat attributes of those sites explained relatively small amounts of this variation. Our results underscore that either eDNA sampling or environmental characterization will require further refinement before eDNA can be used reliably to estimate fish abundance in streams.

Résumé : La possibilité de faire des inférences concernant l'abondance de poissons à partir d'échantillons d'ADN environnemental (ADNe) suscite beaucoup d'intérêt. L'exactitude de telles estimations de l'abondance est toutefois souvent faible et variable pour différentes espèces et dans l'espace. Une amélioration plausible consiste à utiliser des données courantes de surveillance des habitats aquatiques pour y incorporer des attributs qui influencent la dynamique de l'ADNe. Nous avons ainsi évalué les relations entre les concentrations d'ADNe et l'abondance d'ombles à tête plate (*Salvelinus confluentus*), de truites fardées versant de l'Ouest (*Oncorhynchus clarkii lewisi*) et de truites arc-en-ciel (*Oncorhynchus mykiss*) dans 42 sites de cours d'eau dans la région des montagnes de l'Ouest (États-Unis et Canada) et vérifié si l'intégration d'attributs de l'habitat propres au site améliore l'exactitude des estimations d'abondance des poissons. Les concentrations d'ADNe présentent une association positive avec l'abondance de poissons, mais ces relations varient selon l'espèce et le site, et une variation considérable demeure. Des différences aléatoires à l'échelle du site expliquent une bonne partie de cette variation, mais certains attributs de l'habitat dans ces sites expliquent des proportions relativement faibles de cette variation. Nos résultats soulignent le fait que l'utilisation de l'ADNe pour estimer de manière fiable l'abondance de poissons dans les cours d'eau nécessitera d'autres améliorations à l'échantillonnage d'ADNe ou à la caractérisation des conditions ambiantes. [Traduit par la Rédaction]

Introduction

Declines in abundances and distributions of freshwater salmonids across the Northern Hemisphere have resulted in the listing or petition for listing of many species and subspecies as threatened or endangered (Muhlfeld et al. 2018). Quantified estimates and trends of abundance are essential for evaluating listing and recovery decisions, setting conservation priorities, and directing fisheries management plans. However, accurate and precise abundance estimates for populations with low densities and patchy distributions are especially challenging and require substantial effort and monetary resources (Al-Chokhachy et al. 2009; Ham and Pearsons 2000). Furthermore, such estimates (e.g., capture–mark–recapture) incur considerable harassment costs (e.g., handling the species of interest), which can be particularly concerning for imperiled species (Cooke et al. 2011). Conducting such invasive research activities usually requires special permits (Nickum et al. 2004). More efficient and less invasive, yet still effective, methods for monitoring abundance are needed for informed conservation decision-making.

Environmental DNA (eDNA) analysis has recently emerged as a powerful tool to provide quantitative information about the amount of species-specific DNA in an environmental water sample (reviewed in Yates et al. 2019). Aquatic organisms shed cellular and extracellular DNA into the water, where some of it remains suspended and can be collected, analyzed, assigned to targeted taxa, and quantified. Positive relationships exist between the amount of DNA particles in an eDNA sample (hereinafter eDNA concentration)

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and the density or biomass of many aquatic taxa in mesocosms and the field (Lacoursière-Roussel et al. 2016). Analytical frameworks can account for the inherent variability between eDNA concentration and animal density (Chambert et al. 2018), but the strengths of these relationships vary considerably in field settings, with R^2 values ranging from 0.08 to 0.95 (Lacoursière-Roussel et al. 2016; Yates et al. 2019).

The wide range of documented relationships between eDNA concentration and population abundance is probably a result of the complexity of processes that interact to determine the amounts of target DNA available to be sampled rather than intrinsic characteristics of the target species (Barnes and Turner 2016; Deiner et al. 2015; Shogren et al. 2017, 2019). DNA transport, adhesion or deposition, and resuspension are hypothesized to be the primary processes that alter eDNA concentrations over short sample site lengths (e.g., <200 m) because DNA decay rate over such short distances can be negligible (Pilliod et al. 2013; Shogren et al. 2019; Wilcox et al. 2016). Deposition and resuspension have been important determinants in experimental stream mesocosms, wherein a disproportionate amount of DNA can be retained by interaction with the streambed subsurface, substrate, biofilms, and organic matter and then intermittently resuspended back into the water column for further transport downstream. The importance of eDNA retention has been corroborated in field studies by demonstrating that eDNA does not accumulate downriver despite presence of target taxa throughout the river (Laramie et al. 2015*a*; Pilliod et al. 2013; Shogren et al. 2019; Tillotson et al. 2018). Transport distances can also vary, as local habitat and stream power and size interact to create a broad range of potential transport velocities (Pont et al. 2018; Shogren et al. 2017). Disentangling the habitat attributes that affect eDNA dynamics and cause noise in eDNA concentration–target taxa abundance relationships is especially challenging in natural systems.

Here, we describe the relationship between eDNA concentration and metrics of salmonid fish abundance and biomass at 42 stream sites in the Intermountain West (USA and Canada) with low to high densities of target taxa. We also evaluated the effectiveness of including commonly available, standardized salmonid habitat monitoring attributes to moderate the relationships between eDNA concentration and salmonid fish abundance and biomass. Targeted salmonid taxa included bull trout (*Salvelinus confluentus*), westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), and rainbow trout (*Oncorhynchus mykiss*). We addressed two, compound questions: (*i*) Does eDNA concentration increase with salmonid abundance or biomass; and do these relationships vary by salmonid species among and within stream sites? (*ii*) Can variability of eDNA concentrations be further explained by environmental characteristics typically collected during salmonid habitat monitoring; if so, which environmental covariates are consistently associated with lower or higher than expected eDNA concentrations?

Materials and methods

Study areas

Our study included two areas located within the Intermountain West of North America. The first study area contained major tributaries to the Clearwater and Flathead rivers in northwest Montana (USA) that are important spawning and rearing habitat for native bull trout and westslope cutthroat trout (hereinafter Northern Rockies sites; Fig. 1). The climate is typical of the Northern Rocky Mountains, with a snow-dominated precipitation regime including cold, wet winters and springs and warm, dry summers. Seasonal streamflows are dominated by snowmelt, with high flow events during May and June and declining streamflows throughout the summer (Al-Chokhachy et al. 2017).

We surveyed fish populations and collected eDNA samples at 20 Northern Rockies sites, where Montana Fish, Wildlife & Parks (FWP) has conducted long-term fish monitoring (Fig. 1). All sites were visited between 15 July and 9 September 2017 and 2018. Site lengths varied from 100 to 180 m. We assumed DNA released by fish within the site would be detectable at the downstream sampling boundaries (Jane et al. 2015; Wilcox et al. 2016), while providing enough distance for habitat and flow dynamics to potentially influence eDNA concentrations (Shogren et al. 2017; Tillotson et al. 2018; Wilcox et al. 2016).

We selected sample sites to represent a range of bull trout and westslope cutthroat trout abundances based on past monitoring data; most populations were a mix of resident and migratory life histories. Sample sites occurred across a range of stream orders, morphologies, and channel types typical within the distribution of bull trout (Al-Chokhachy et al. 2010). Gradients ranged from 0.06% to 5.00%, wetted widths from 3.2 to 11.6 m, and discharge from 0.06 to 2.47 $m^3 \cdot s^{-1}$ (refer to online Supplementary material, Table S1[1]). In addition to native bull trout and westslope cutthroat trout, native sculpin (*Cottus* spp.) and non-native brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) were present at varying levels.

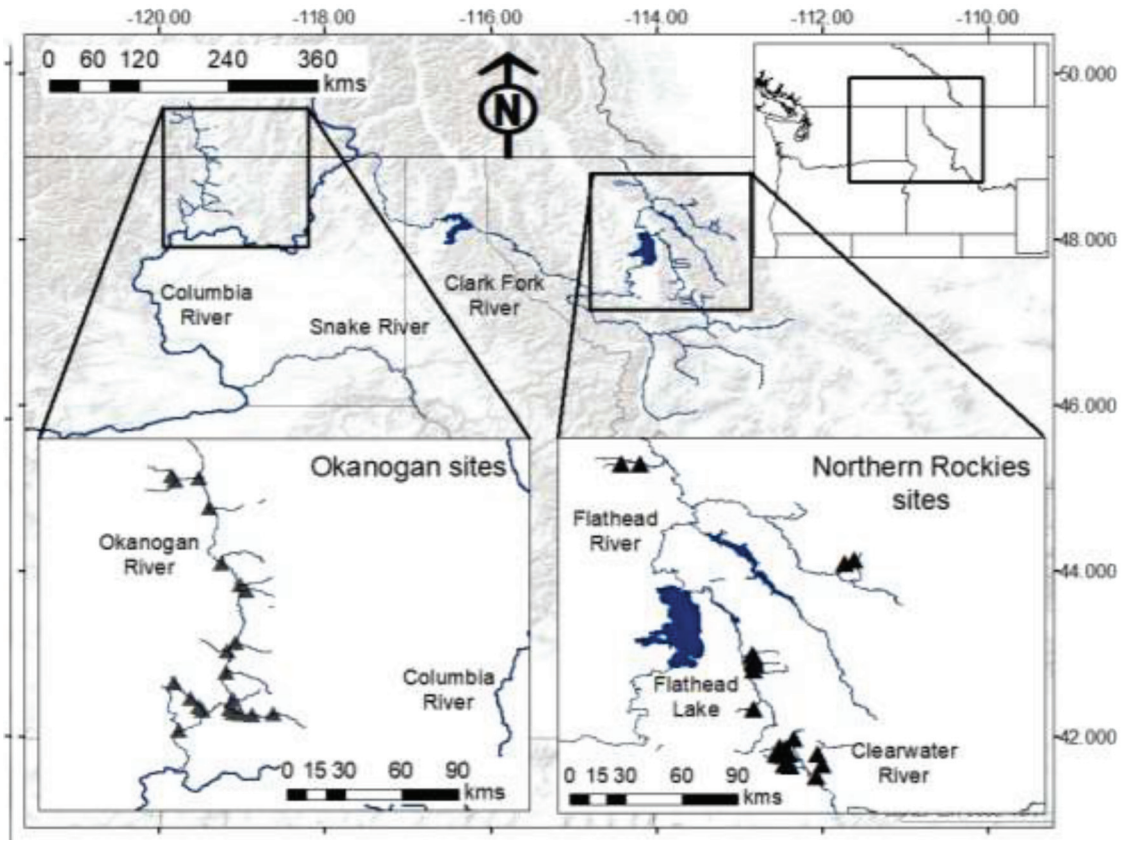
Our second study area contained major tributaries of the Okanogan River in north-central Washington, USA, and southern British Columbia, Canada (hereinafter Okanogan sites; Fig. 1), which are important spawning and rearing habitat for rainbow trout and its anadromous form steelhead. The Okanogan River runs from its source at Lake Okanogan, near Penticton, British Columbia south to its confluence with the Columbia River near Bridgeport, Washington. Climate parallels that of the Northern Rockies region, but with warmer temperatures and less snowfall at lower elevations during the winter. Similarly, the seasonal streamflows in the Okanogan River peak in May and June and taper throughout the summer. In addition to rainbow trout, the Okanogan basin also supports Chinook salmon (*Oncorhynchus tshawytscha*), native sculpin, and a suite of non-native fishes, including brook trout and bass (*Micropterus* spp.). The Okanogan sites were selected based on an annual monitoring strategy implemented by the Confederated Tribes of the Colville Reservation – Okanogan Basin Monitoring and Evaluation Program that is typical of the habitat monitoring that occurs throughout the Columbia River basin (e.g., ISEMP–CHaMP 2017). All sites were part of an effort to assess the status and trends of steelhead, as well as the types and condition of available habitats throughout the basin (OBMEP 2017). A total of 22 sites on 13 tributary streams were sampled. Site lengths averaged 150 m and ranged from 95 to 193 m. All sites were visited between 8 September and 20 October 2016, when gradients ranged from 0.4% to 5.2%, wetted widths from 1.4 to 6.2 m, and discharge from 0.003 to 0.252 $m^3 \cdot s^{-1}$ (Supplemental Table S2[1]).

Field sampling

Our field sampling approach included initial habitat surveys, eDNA collection, and fish abundance surveys. Sampling occurred during baseflow conditions (July through October) before many migratory bull trout and brook trout occupied the streams to spawn in autumn and after cutthroat trout and rainbow trout spawned during spring, thus avoiding confounding factors associated with greater variability in fish biomass and age, as well as eDNA sampling during reproductive periods (e.g., the introduction of reproductive genetic materials; Tillotson et al. 2018). Sampling during baseflow conditions also allowed us to characterize stream habitat attributes more accurately.

[1]Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2020-0182.

Fig. 1. Map of the two study areas located within the Intermountain West (insets), including parts of the Okanogan Basin in Washington (USA) and British Columbia (Canada) and the Northern Rocky Mountains in northwest Montana (USA), with locations of sample sites (triangles) and major rivers on inset maps. The map was developed using Esri ArcGIS 10.7.1; basemaps are from Esri, and stream network layers are from the US Geological Survey's National Hydrography Dataset (www.nhd.usgs.gov).



Stream habitat attributes

Standard fisheries habitat monitoring protocols were used to assess habitats at each site. Habitats at the Northern Rockies sites were assessed 2 to 4 weeks prior to eDNA and fish sampling using PacFish/InFish Biological Opinion Monitoring Program protocols (Archer et al. 2016). For the Okanogan sites, we included the most recent habitat survey data (2013 to 2017) collected using protocols in the Monitoring Strategy for the Upper Columbia Basin (Hillman and Board 2004) with reference to the methods of the Collaborative Systemwide Monitoring & Evaluation Program and the Pacific Northwest Aquatic Monitoring Partnership. We assumed that these recent habitat data adequately described conditions during fish and eDNA sampling, though we did not test this assumption. Specific methods are described in Okanogan Basin Monitoring and Evaluation Program field manuals: Okanogan Basin Physical Habitat Monitoring Version 2.2. (Arterburn et al. 2007), Snorkel Survey Methodology Final Draft (The Colville Confederated Tribes et al. 2007), and Water Quality Sampling Protocols (The Colville Confederated Tribes et al. 2005).

Habitat assessment methods differed slightly between the Northern Rockies and Okanogan sites, but assessments produced a subset of comparable data on variables frequently associated with salmonid abundance (Table 1). Variables recorded in both regions were site length, gradient, water temperature, discharge, wetted area, percent pool, substrate composition, and large woody debris amount. Variables collected only at Northern Rockies sites were residual pool depth, average reach velocity, and roughness (described in Supplemental Material 2[1]). Variables collected only at Okanogan sites were water conductivity, canopy cover, and percent area of the following habitat categories: cascades or falls, cobble–riffles, rapids, glides, beaver ponds, and dry areas.

eDNA field sampling

Environmental DNA water samples were collected immediately before any staff or equipment (e.g., block nets) entered the water to sample fish abundances in both study areas. Field methods differed slightly between the study areas. In the Northern Rockies, we collected three to four eDNA samples at both the upstream (hereinafter, $eDNA_{above}$) and downstream (hereinafter, $eDNA_{below}$) boundaries of each site, 2–4 weeks after habitat assessments. We collected the initial sample at the farthest downstream boundary of the site and collected each additional sample ~1 m upstream from the previous sample. We then repeated this process for the $eDNA_{above}$ samples, at the farthest upstream boundary of the site. Samples were collected using a peristaltic pump, drawing 5 L of stream water through a 47-mm diameter, 1.5-μm pore glass fiber filter (Carim et al. 2015). We also collected a field negative control (1 L deionized water) at each of seven sites to assess potential contamination of sample collection equipment. Filters were folded with single-use forceps and individually sealed in sterile plastic bags with silica desiccant for preservation (Carim et al. 2015). We temporarily stored samples in a cooler and later transferred them to refrigerated, dark storage. All samples were transported to the US Forest Service National Genomics Laboratory (Missoula, Montana) within 14 days of collection and placed in a −80 °C freezer until sample extraction and analysis.

At the Okanogan sites, sample filtration and preservation followed the protocol described in Laramie et al. (2015*b*). We simultaneously collected three 500-mL water samples in separate,

Table 1. Habitat characteristics measured at Northern Rockies and Okanogan sites, following standard fisheries habitat assessment protocols.

Category	Attribute	Northern Rockies	Okanogan	Description
Substrate	Large woody debris	X	X	Total number of pieces of submersed wood at least 0.5 m in length and 0.05 m in width
	D_{50}	X	—	Median particle size
	Proportion substrate type (fines, sand, etc.)	—	X	Proportion of total reach length characterized by the dominant substrate type (fines, sand, etc.)
Mesohabitat	Wetted width	X	X	Average width of multiple transects
	Proportion pools	X	X	Sum of the pool lengths divided by total site length
	Proportion habitat type (cascade, riffle, etc.)	—	X	Proportion of total reach length characterized by hydrologic habitat type (cascade, riffle, etc.)
	Residual pool depth	X	—	Average difference between the maximum pool depth and pool tail depth across all pools
	Canopy cover	—	X	The mean number of densiometer grid intersection points covered by vegetation
Flow dynamics	Discharge	X	X	Measured at the downstream boundary of each reach
	Gradient	X	X	Elevation change of the water surface between the upstream and downstream extent of each site
	Average reach velocity	X	—	Time for salt dilution tracer to reach downstream extent of each site
	Roughness	X	—	Manning's equation
Water chemistry	Temperature	X	X	Recorded at time of eDNA sample collection
	Conductivity	—	X	Water quality parameter measuring the relative concentration of ions (measured in μS)
Other	Fish distributions skewness	X	—	No. of fishes captured in 10-m segments during the first pass

sterile Whirl-Paks at both the upstream and downstream extents of each site (i.e., where block nets were later installed) for a total of six samples per site. The samples were collected at the surface along a transect perpendicular to streamflow at the left bank (~10 cm from the streambank), right bank (~10 cm from the streambank), and center. We collected a field negative control (500 mL distilled water) and filtered it streamside along with the field samples at each site to assess potential contamination of sample collection equipment. Samples were filtered using a peristaltic pump that pulled sample water through a 47-mm diameter, 0.45-μm pore nitrocellulose filter. Filters were folded with single-use forceps and placed into separate 2.0-mL DNase-free cryo-vials with 200-proof (100% molecular-grade) EtOH for preservation.

Fish population data

Fish sampling began immediately after eDNA sample collection in both areas but differed based on local agency methods and monitoring protocols. In the Northern Rockies sites, we used multiple-pass backpack electrofishing methods in collaboration with Montana FWP and estimated species-specific abundance using depletion methods (Peterson et al. 2004). We deployed block nets for closure at the downstream and upstream boundaries. Fish captured in each pass were held in separate live wells until all passes were completed and anesthetized with Aqui-S 20E (AQUI-S, New Zealand) until they lost equilibrium. We identified individuals to species, measured the total length (mm) and mass (g) of each fish when possible, placed fish in a pass-through recovery tank within the river after measurements were completed, and returned fish proximate to points of capture after they regained equilibrium. We could not estimate total abundances at all sites because of low densities and patchy distributions; consequently, we used relative abundance, defined as the total number of fish of a species captured during the first pass, in our analyses. Estimated total and relative abundances of bull trout (adjusted $R^2 = 0.91$, $n = 11$, $P < 0.001$) and westslope cutthroat trout (adjusted $R^2 = 0.97$, $n = 16$, $P < 0.001$) were highly correlated at the subset of sites where we could estimate total fish abundances.

We used the "FSA" package in R (Ogle et al. 2018) to estimate fish biomass at each site. First, we calculated length–weight regressions based on the subset of fish with both total length and mass measurements. We then estimated mass for each individual fish by incorporating fish lengths into the following regression equations with intercept and slope coefficients and a bias correction: bull trout mass = $\exp(-11.1881 + 2.8960 \times \ln(\text{total length, mm})) \times 1.0039$; westslope cutthroat trout mass = $\exp(-11.5333 + 2.9772 \times \ln(\text{total length, mm})) \times 1.0580$.

We recorded the species and numbers of fish collected within 10-m stream segments during the first electrofishing pass to evaluate the extent to which fish positions tended to cluster upstream of the eDNA sampling point. We used the number of fish in each 10-m bin to estimate a site skewness value. We hypothesized that observed eDNA concentrations would be less than expected when fish distributions clustered near the upstream edge of the 10-m stream segment (i.e., left-skewed). We assumed that the first pass best resembled the actual fish distribution during eDNA sampling.

At the Okanogan sites, we installed block nets at the upstream and downstream extents of each site to ensure a closed population and then conducted two-pass mark–recapture backpack electrofishing surveys to estimate rainbow trout abundance. All trout captured during the first electrofishing pass were measured (fork length, mm), marked with a top caudal fin clip, and released near their initial capture locations. A second pass was conducted 3 h later. During the second pass, all fish were examined for a mark. We calculated site-level abundance estimates (OBMEP 2017) using the Lincoln–Peterson mark–recapture model, as modified by Chapman (Chapman 1954), bearing the standard assumptions: (*i*) closed population; (*ii*) consistent sampling efforts; (*iii*) equal capture probability of marked and unmarked fish; (*iv*) marked fish randomly mixed within population; and (*v*) detectable marks. Trout biomass at each site was estimated from individual length measurements of fish collected during electrofishing using a standard length–weight equation for rainbow trout in lotic systems; $\log_{10}(\text{weight, g}) = -5.023 + 3.024 \times \log_{10}(\text{total length, mm})$ (Simpkins and Hubert 1996). Biomass estimates were the sums of estimated weights of all fish handled during first passes.

Lab procedures to quantify eDNA concentration

Species-specific sets of primers and probes were used to amplify targeted trout mitochondrial DNA. Molecular assay sequences and

qPCR reaction conditions are detailed in Dysthe et al. (2018) for bull trout and Wilcox et al. (2015) for westslope cutthroat trout and rainbow trout. For Northern Rockies samples, DNA extraction and amplification were performed at the US Forest Service National Genomics Laboratory (Missoula, Montana, USA) in an eDNA-explicit laboratory and in accordance with the protocols outlined in Dysthe et al. (2018). Triplicate qPCR analyses were run for each sample, and results were reported as mean copy numbers per litre of water sampled of the triplicate wells. For Okanogan samples, DNA extraction and amplification were performed at the US Geological Survey Forest and Rangeland Ecosystem Science Center — Pacific Northwest Environmental DNA Laboratory (Boise, Idaho, USA) in isolated, eDNA-explicit rooms and in accordance with the guidelines outlined in Goldberg et al. (2016). DNA extraction protocols followed those described by Laramie et al. (2015*a*). For all samples, qPCR analyses were run in triplicate, and picograms per litre of water sampled was reported as the mean estimate for triplicate wells. We acknowledge that eDNA concentrations were minimum estimates of the actual amount of target DNA in the water that was sampled because they did not account for the DNA lost during each stage of the workflow (e.g., filtration, storage, extraction). Cq values were determined using a single threshold and baseline subtracted curve fit on Bio-Rad CFX Maestro 1.1 software version 4.1.2433.1219 (Bio-Rad Laboratories, Inc., California, USA). Negative controls were used throughout DNA extraction and qPCR analysis for both the Northern Rockies and Okanogan analyses. No sample contamination was detected at any point in this study.

Statistical analysis

We used generalized mixed linear models (GLMMs) to evaluate the relationship between eDNA concentration and fish abundance metrics. We initially considered two separate measures of eDNA concentration. First, we used $eDNA_{below}$ as the measure of eDNA concentration at the site scale (e.g., Doi et al. 2017). Second, we calculated ΔeDNA as $eDNA_{below}$ minus $eDNA_{above}$ and, following Wilcox et al. (2016), corrected for downstream persistence (P) for any site length that was >100 m:

$$P = \text{eDNA}_{\text{above}} \times p^{1(\text{site length}/100\,\text{m})}$$

where p^1 is an estimated constant persistence rate (0.63) or proportion of eDNA remaining after 100 m.

This second measure was used in an attempt to limit our inferences to the study site, since eDNA particles can be transported long distances. However, ΔeDNA values were significantly correlated with $eDNA_{below}$ (R = 0.99, $P < 0.01$), so we used the simpler $eDNA_{below}$ for analyses (hereinafter, just eDNA). We also considered four separate measures of fish abundance: abundance (target fish captured in the first pass in the Northern Rockies; estimated total target fish in the Okanogan), estimated density (abundance·wetted area^{-1}), biomass (total target fish biomass captured in the first electrofishing pass), and first-pass biomass density (biomass·m^{-2}).

We characterized the relationships between eDNA concentration and fish abundance separately for each target fish species (Models 1*a* and 1*b*; see below). We used only the Northern Rockies data set to test whether the relationships between eDNA concentration and abundance metrics differed between the two trout species co-occurring at the same sites (Models 2*a* and 2*b*; see below). Both analyses used the modeling protocols recommended by Zuur et al. (2009) and Grueber et al. (2011). First, we standardized all fish abundance metrics to a mean of 0 and a standard deviation of 0.5 and $\log_{10}$-transformed eDNA concentration. Second, we evaluated the appropriate variance structure using likelihood ratio tests to compare a linear regression fit using base R (R Core Team 2013) with a mixed effect model with the random intercept of site using the "nlme" package (Pinheiro et al. 2017). We lacked enough observations to test single species models that included random effect terms for slope. We used an analysis of variance (ANOVA) and the reported χ^2 and Akaike information criteria for small sample size (AIC_c) to test whether inclusion of random terms improved model fit. We then used models with the optimal variance structure to evaluate which abundance metrics were most supported by the data (i.e., ΔAIC_c). These models were fit using the "lme4" (Bates et al. 2015) and "lmertest" packages in R (Kuznetsova et al. 2017), AIC_c values were estimated using the "bbmle" package in R (Bolker 2014), and we evaluated the statistical assumptions from the most-supported model using the "performance" package in R (Lüdecke et al. 2019). We report model results using scaled parameter estimates of the top models, though figures display back-transformed fish abundance metric values for ease of interpretation. General model structure for trout species i, at site j in sample k was

$$(1a) \quad \text{eDNA concentration}_{ijk} \sim \text{Abundance metric}_{ij}$$

$$(1b) \quad \text{eDNA concentration}_{ijk} \sim \text{Abundance metric}_{ij} + (1|\text{Site}_{ij})$$

$$(2a) \quad \text{eDNA concentration}_{ijk} \sim \text{Abundance metric}_{ij} \times \text{Fish species}_{ij}$$

$$(2b) \quad \text{eDNA concentration}_{ijk} \sim \text{Abundance metric}_{ij} \times \text{Fish species}_{ij} + (1|\text{Site}_{ij})$$

where fixed effects included "Abundance metric" and "Fish species", and random effects included "Site".

Next, we tested whether any habitat variables were consistently associated with the residuals from the top models of each analysis. For the Northern Rockies sites, we also assessed whether fish distribution skewness was associated with residuals. First, we scaled all habitat variables to a mean of 0 and a standard deviation of 1. We then calculated the Pearson correlation coefficients between the residuals, the scaled habitat variables, and the skewness of the fish distributions. The three habitat variables with the largest correlation coefficients were then included as additive fixed effects in the previous top models to construct a species-specific and Northern Rockies-specific global models. Next, we used the "drop1" function in the "lme4" package to identify a reduced model that only retained supported and significant single fixed-effect habitat terms. Finally, we used AIC_c values to compare our initial top models without habitat covariates with the following models: global model, reduced model, and models that only included a single habitat covariate from the global model. To evaluate whether the inclusion of habitat or skewness variables improved fit, we compared the AIC_c and marginal R^2 or conditional R^2 values, which were estimated using the "model_performance" command in the "performance" package. All statistics were done in R version 3.6.1.

Results

Fish captures and eDNA concentration

Bull trout and westslope cutthroat trout were both captured in 16 of the 20 Northern Rockies sites. Only bull trout were captured in one site, and only westslope cutthroat trout were captured in three sites. Rainbow trout were captured at all Okanogan sites. Target fish eDNA was detected in all sites, including the Northern Rockies sites where bull trout or westslope cutthroat trout were not captured. Summary statistics of captured fish and target fish eDNA are provided in Table 2.

Table 2. Means (±1 SE) and ranges of fish abundances, biomasses, and lengths and eDNA quantities of bull trout and westslope cutthroat trout in the Northern Rockies sites and rainbow trout in the Okanogan sites.

	Abundance		Biomass			
Species	Fish	Fish·m^{-2}	g	g·m^{-2}	Length (mm)	eDNA concentration*
Bull trout	16±8 (0–84)	0.01±0.01 (0–0.06)	468±220 (0–2162)	0.45±0.23 (0–2.36)	119.4±2.2 (40–550)	744±316 (20–3051)a
Westslope cutthroat trout	22±10 (0–110)	0.04±0.02 (0–0.23)	398±131 (0–1375)	0.58±0.26 (0–2.84	120.6±1.7 (16–270)	681±295 (87–3251)a
Rainbow trout	360±69 (12–1254)	0.72±0.18 (0.02–3.69)	2234±517 (29–8787)	4±1 (0.1–9.7)	92.7 0.6 (32–330)	1.0 ± 0.2 (0.02–4.3)b

*eDNA collected at the downstream boundary of each study site are reported as copies (copies·L^{-1}) (a) or as picograms (pg·L^{-1}) (b) and describe the minimum estimates of the actual amount of target DNA in the water that was sampled.

Table 3. Mixed-model output of the top models describing the relationship between eDNA concentration and fish abundance metrics analyzed separately for bull trout, westslope cutthroat trout, and rainbow trout.

	$\log_{10}$ (eDNA concentration)					
	Bull trout		Westslope cutthroat trout		Rainbow trout	
Predictors	Estimate (CI)	*P*	Estimate (CI)	*P*	Estimate (CI)	*P*
Intercept	2.40 (2.16–2.63)	<0.01	2.71 (2.53–2.89)	<0.01	2.81 (2.63–2.99)	<0.01
Biomass	0.91 (0.43–1.38)	<0.01	0.48 (0.12–0.84)	0.01	0.48 (0.11–0.84)	0.01
Random effects						
σ^2	0.04		0.04		0.04	
$\tau_{00\ Site}$	0.28		0.16		0.18	
ICC	0.88		0.80		0.83	
N_{Site}	20		20		22	
Observations	65		65		65	
Marg. R^2, cond. R^2	0.39, 0.93		0.22, 0.84		0.22, 0.87	

Note: Estimates are shown with confidence intervals in parentheses. Random effects components include residual error (σ^2), variation explained by random effect of site ($\tau_{00\ Site}$), intraclass correlation coefficient (ICC), the number of sites (N_{site}), the number of observations, and the marginal (marg.) and conditional (cond.) R^2 measures.

Single species analyses: eDNA concentration – fish abundance metric relationships

The variance structure that included the random intercept of site was more supported than a simpler linear model in all separate target species analyses ($\Delta AIC_c > 40$, $P < 0.001$). Using this variance structure, we showed that all four abundance metrics were plausible for each target species ($\Delta AIC_c < 5.6$; Supplemental Table S3[1]). The model including biomass was the most supported in describing eDNA concentration for bull trout, followed by abundance ($\Delta AIC_c = 0.3$). The model including biomass was also the most supported for westslope cutthroat trout, followed by biomass density ($\Delta AIC_c = 3.7$). Models with biomass or biomass density were most supported for rainbow trout ($\Delta AIC_c < 0.3$). To facilitate interspecies comparisons, we report just the results for biomass models and conducted subsequent analyses using the biomass models.

The intercepts and slope estimates describing the relationship between log-transformed eDNA concentration and scaled biomass were significantly greater than 0 but varied by species (Table 3 and Fig. 2). The rainbow trout relationship had the highest mean intercept, whereas the relationship for bull trout had the highest mean estimated slope (Table 3). For each gram increase in fish biomass, the mean DNA quantities (±95% CI) increased by 8 (2–23) copies·L^{-1} for bull trout, 3 (1–7) copies·L^{-1} for westslope cutthroat trout, and 3 (1–7) pg·L^{-1} for rainbow trout. The proportion of the variance explained by the random intercept of site was high for relationships of all species and ranged from 0.80 to 0.88 (Table 3). Relationships for each trout species appeared to be leveraged by a few sites with extreme abundance metric values. Though these sites did not meet criteria for removal (e.g., Cook's distance > 1), we conducted post hoc analyses without these sites to better assess how they influenced the modeled relationships. For the bull trout relationship, the mean (±1 SE) biomass slope estimate increased from 0.91 (0.24) to 1.65 (0.32). For the westslope cutthroat trout relationship, the slope increased from 0.48 (0.18) to 0.68 (0.40) but was not statistically greater than zero. For the rainbow trout relationship, the slope changed minimally from 0.48 (0.19) to 0.43 (0.18). We used the initial models that included all sites for further analyses.

Habitat variables and fish distribution skewness values did not have high correlations with residuals from the aforementioned top models for any fish species. For bull trout, correlations between habitat covariates and the residuals from the top model ranged from −0.35 to 0.50. Discharge ($R = 0.41$), percent pool ($R = 0.44$), and large woody debris count ($R = 0.50$) had the highest correlations. The drop1 function indicated that only discharge should be retained ($F = 4.81$, $P = 0.04$). The global model had similar support as the reduced model with only biomass and discharge ($\Delta AIC_c = 1.4$), though both of these models had more support than the simplest model with only biomass ($\Delta AIC_c = 7.6$). The conditional R^2 values (i.e., amount of variation explained by both the fixed and random effects) of the models were similar among these three models (0.92–0.93), but the marginal R^2 values (i.e., amount of variation explained by only the fixed effects) improved from 0.34 in the simplest model to 0.52 in the drop1 model to 0.62 in the global model. These higher marginal R^2 values suggest that AIC_c-based assessments may be hyperconservative and over-penalize more complex models. We provide the global model results in Table 4.

For westslope cutthroat trout, correlations between habitat covariates and the residuals from the top model ranged from −0.12 to 0.15. Large woody debris count ($R = 0.15$), percent pool ($R = 0.14$), and roughness ($R = -0.12$) had the highest correlations. The drop1 function indicated that no habitat covariates should be retained. The top model with only biomass was more supported for explaining eDNA concentration than the global model ($\Delta AIC_c = 7.1$).

For rainbow trout, correlations between the habitat covariates and the residuals from the top model ranged from −0.49 to 0.47.

Fig. 2. Predicted (solid line) and observed (points) DNA concentration relationships with bull trout, westslope cutthroat trout, and rainbow trout biomass. Shaded bands indicate the 95% confidence intervals of the predicted lines.

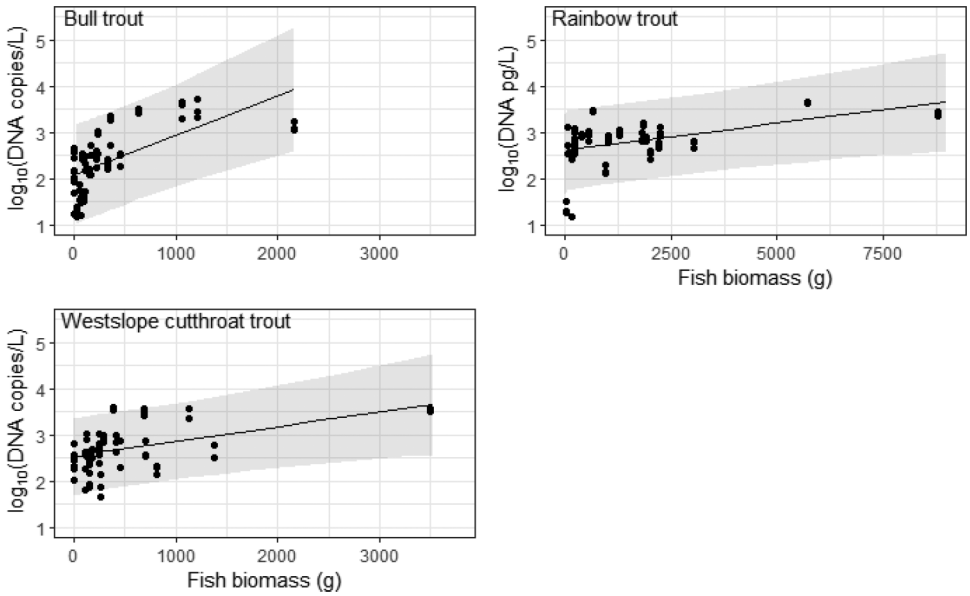


Table 4. Mixed-model output of the top models describing the relationship between eDNA concentration, fish abundance metrics, and habitat (when supported) analyzed separately for bull trout, westslope cutthroat trout, and rainbow trout.

	$\log_{10}$ (eDNA concentration)					
	Bull trout		Westslope cutthroat trout		Rainbow trout	
Predictors	Estimate (CI)	*P*	Estimate (CI)	*P*	Estimate (CI)	*P*
Intercept	2.34 (2.15–2.52)	<0.01	2.71 (2.53–2.89)	<0.01	2.83 (2.67–3.00)	<0.01
Biomass	0.70 (0.33–1.07)	<0.01	0.48 (0.12–0.84)	0.01	0.40 (0.07–0.73)	0.02
Discharge	0.22 (0.02–0.41)	<0.01	—		—	
LWD count	0.14 (−0.07–0.34)	0.18	—		—	
Percent pool	0.14 (−0.07–0.35)	0.20	—		—	
Cobble–riffle	—		—		−0.25 (−0.44 to −0.07)	0.01
Random effects						
σ^2	0.04		0.04		0.04	
$\tau_{00\ Site}$	0.14		0.16		0.14	
ICC	0.79		0.80		0.79	
N_{Site}	18		20		21	
Observations	59		65		62	
Marg. R^2, cond. R^2	0.62, 0.92		0.22, 0.84		0.39, 0.87	

Note: See Table 3 for description of random effects terms.

The proportions of cobble–riffle habitat ($R = -0.49$), hardpan substrate ($R = -0.36$), and pool habitats ($R = 0.31$) had the highest correlations. The drop1 function indicated that no habitat covariates should be retained. The top model with only biomass was similarly supported as a model that included biomass and the proportion of cobble–riffle habitat ($\Delta AIC_c = 1.1$). The conditional R^2 values of these models were similar (0.87), but the marginal R^2 values improved from 0.21 in the simplest model to 0.39. Both of these models had more support than models that included the other habitat covariates ($\Delta AIC_c = 3.5$) or the global model ($\Delta AIC_c = 7.7$).

Multiple species analyses: eDNA concentration – fish abundance metric relationships

For co-occurring bull trout and westslope cutthroat trout, a variance structure that included the random intercept of site and the random slope of fish species was more supported than a simpler linear model ($\Delta AIC_c = 127$) or a random intercept only model ($\Delta AIC_c = 52$). Using this variance structure, models that included biomass and abundance were similarly supported ($\Delta AIC_c = 0.4$) and received more supported than other abundance metrics ($\Delta AIC_c > 7.4$). For consistency with previous analyses, we report model results for just biomass (Table 5). The biomass × fish species interaction was not significant, but the intercept and the biomass and fish coefficient estimates were significantly greater than 0. Westslope cutthroat trout had a higher intercept than bull trout by ~2 copies DNA·L^{-1}. The random intercept of site explained 46% of the variance, and the random slope of fish species explained 48% of the variance.

Discussion

Our study has one of the numerically largest (i.e., sample size) and spatially broadest data sets yet to evaluate the relationship between eDNA concentration and target fish species abundance, especially in lotic systems. We found that the eDNA

Table 5. Mixed-model output of the top model describing the relationship between eDNA concentration, fish abundance metrics, and habitat (when supported) analyzed jointly for bull trout and westslope cutthroat trout in Northern Rockies sites.

	$\log_{10}$ (eDNA concentration)		
Predictors	Estimate	CI	*P*
Intercept	2.40	2.16–2.63	<0.01
Biomass	0.89	0.45–1.33	<0.01
Fish species	0.31	0.07–0.56	0.01
Biomass × Fish species	−0.42	−1.00–0.15	0.15
Random effects			
σ^2	0.04		
$\tau_{00\ \text{Site}}$	0.28		
$\tau_{11\ \text{Site.FishWestslope}}$	0.29		
$\rho_{01\ \text{Site}}$	−0.71		
ICC	0.85		
N_{Site}	20		
Observations	130		
Marg. R^2, cond. R^2	0.37, 0.90		

Note: See Table 3 for description of random effects terms. Additional components include random slope variance (τ_{11}) and the random slope-intercept correlation (ρ_{01}).

concentration – fish abundance metric relationships were positive but varied among the three trout species sampled at our 43 sites, even when target species co-occurred in the same site. The slope of the eDNA relationship with bull trout biomass was about two times larger than for the relationships with westslope cutthroat trout and rainbow trout. Inclusion of site-specific habitat attributes did relatively little to improve these eDNA relationships, even though site-level effects were significant. Our results underscore that either eDNA sampling strategies or the methods we use to characterize environmental conditions relevant to eDNA production, degradation, and redistribution require further refinement. This is especially important if eDNA-based abundance trends are to be used to inform conservation priorities.

For brevity, we only reported relationships between eDNA concentration and fish biomass. However, it is important to underscore that, like Yates et al. (2019), we found no evidence (i.e., ΔAIC_c values) that biomass was more strongly associated with eDNA concentration than abundance, density, or biomass density. Slope estimates describing the relationship between eDNA concentration and fish biomass (and other abundance metrics) were significantly positive, but similar to other studies the relationships had wide confidence intervals and were leveraged by several outlier sites (Doi et al. 2017; Lacoursière-Roussel et al. 2016; Pilliod et al. 2013; Wilcox et al. 2016). Marginal R^2 values (i.e., variance explained by the abundance-metric fixed effect) ranged from 0.22 to 0.39, which are lower than 95% of the linear-regression-estimated R^2 values reported from 19 natural ($R^2 \sim$ 0.50–0.65) and laboratory ($R^2 \sim$ 0.75–0.85) studies in Yates et al. (2019). However, our relationships surpassed many of those reported in Yates et al. (2019) once the random effect of site was accounted for, with conditional R^2 values ranging from 0.84 to 0.93. This suggests that there are either environmental characteristics of sites or characteristics of the fish communities occupying those sites that **could** help better predict fish abundance from eDNA data.

Despite the ability of our models to account for most of the variation, we still documented considerable differences in eDNA concentrations for similar fish biomass values and similar eDNA concentrations for a broad range of fish biomass values, including the outlier sites (Fig. 2). For example, sites with bull trout biomass values of ~100 g had eDNA concentrations that ranged from 95 to 575 copies·L^{-1}, whereas sites with bull trout eDNA concentrations of ~575 copies·L^{-1} had biomass values that ranged from 0 to 448 g. This broad range of inference between eDNA concentrations and fish abundance illustrates the challenges of using eDNA methods for estimating trout abundance. Additionally, the estimated eDNA concentrations when target fish abundance was zero (i.e., the intercept) were significantly positive and equated to 251–512 copies·L^{-1} for bull trout and westslope cutthroat trout (Table 2). Thus, it may be difficult to discern when eDNA concentrations are signals of low-level abundance or just noise from eDNA originating from a nonpertinent source (e.g., upstream of the study site or released from the substrate). Confidence in discerning signal from noise is further complicated by the assays' limits of quantification, the lowest amount of DNA in a sample that can be quantitatively determined with high precision (Klymus et al. 2020). For assays used in this study, the limits of quantification under ideal conditions (e.g., molecular-grade water) is about 10 copies per reaction, which scales to 100 copies·L^{-1}, but is probably much larger when challenged with environmental conditions typical of a field water sample (Sepulveda et al. 2020). These results provide strong evidence that eDNA concentrations cannot be used to discern fine differences in fish abundance metrics at our study sites and call into question whether or not it is even possible to reliably discern very small from very large fish abundance metric values (Fig. 2). These limitations are especially troubling for any monitoring program needing to detect declining trends in fish abundance for imperiled populations. Whereas these poorly understood effects confound quantitative abundance estimates, they do not seem to have as great an effect on presence–absence assessments (e.g., occupancy models) using eDNA detection methods. This is likely due to the overall sensitivity of the method, which can provide excellent detection probabilities, but may actually suffer from spatial sensitivity in the quantitative realm (i.e., noise) when point estimates are needed.

Ultimately, eDNA-inferred point estimates of fish abundance may not be suitable as a direct-replacement alternative for traditional methods in streams. There are fundamental differences between eDNA and traditional methods that are difficult to reconcile. For example, fish are trapped between block nets when electrofishing, whereas eDNA is not. Traditional methods provide an estimate specific to one point in time and space (e.g., a reach at the time of sampling), whereas eDNA analyses likely sample DNA from much larger spatiotemporal scales. Thus, electrofishing and eDNA analyses are likely complementary methods that answer different questions. If the management objective is to make inferences about the larger fish population in an open, linear stream system where fish and their habitats are heterogeneously distributed, then which method results in samples that are more reflective of the population remains an unanswered question. Indeed, the disconnect between stream research conducted at small spatiotemporal scales and fish life histories that occur at larger spatiotemporal scales was underscored almost 20 years ago when Fausch et al. (2002) implored researchers to measure stream fish at appropriate scales (i.e., "riverscapes").

Inclusion of site as a random intercept greatly improved model fit for all three trout species, suggesting that there are environmental attributes that are consistently associated with lower or higher than expected eDNA concentrations. Indeed, many other studies have found clear indication that environmental conditions influence eDNA dynamics (Jerde et al. 2016; Seymour et al. 2018; Shogren et al. 2017; Tillotson et al. 2018). Yet, we found minimal support for including available habitat attributes into models. The amount of pool habitat was the only environmental attribute that had higher correlations with model residuals for all three trout species, with increases in pool habitat associated with higher than expected eDNA concentrations (i.e., positive residuals). This correlation is counter to what has been found in

experimental and natural systems, where habitat attributes that slow the downstream transport of eDNA allow for increased DNA degradation or storage (Jerde et al. 2016; Shogren et al. 2017), so should result in negative residuals. Alternatively, pool habitat may be used by high densities of fish, especially in the relatively shallow rivers (sampled at baseflow) of the Intermountain West, and indeed store eDNA but also consistently release large reservoirs of stored eDNA downstream (Jerde et al. 2016). For our study, pool habitat was only retained as an informative fixed effect for bull trout, though coefficient estimates overlapped zero. The lag time between habitat assessments and eDNA and fish sampling may have contributed additional noise to these models, but we suspect it was minimal because all assessments and sampling occurred during baseflow conditions.

Co-occurrence of bull trout and westslope cutthroat trout in a common environment at 20 sites provided a strong empirical test of the importance of environmental attributes to the amount of target eDNA captured and amplified from co-occurring species. If environmental attributes are a dominant control on eDNA dynamics, then eDNA concentration – fish abundance relationships between bull trout and westslope cutthroat trout should have been similar at a site. Our results did not support this prediction. The most-supported variance structure for our multispecies models included a random slope that varied by fish species and a random intercept that varied by site. Additionally, the model residual–habitat attribute correlations differed for bull trout and westslope cutthroat trout. For example, the correlation of bull trout residual values with pool habitat and large woody debris was high (R = 0.44 and 0.50, respectively), while the same correlations were lower for westslope cutthroat trout (R = 0.14 and 0.15, respectively). These interspecies within-site differences lead to the hypothesis that the eDNA concentration – abundance relationship is influenced more by how a target species population or group of individuals interact with the habitat than by the habitat itself. In our case, resident bull trout are known to move minimally, use microhabitats in cover (e.g., large wood, undercut bank) or closest to the streambed where current velocities are low, and be most active at night (Al-Chokhachy et al. 2010; Muhlfeld and Marotz 2005; Nakano et al. 1998). In contrast, cutthroat trout use microhabitats closer to the stream surface where current velocities are higher, move further and more frequently for foraging, use less cover, and are more active during the day (McIntyre and Rieman 1995; Nakano et al. 1998). It is likely even more nuanced than these general natural history descriptions since the size or age structure of a group of fishes may interact with the environment differently than another group of fishes from the same target species (Levi et al. 2019). Thus, DNA shed by these species variably interacts with different micro- and mesohabitat attributes. These differences may be further exacerbated by interspecies variation in DNA shed rates and nocturnal versus diurnal foraging activity, given that metabolic rates and activity of organisms are known to influence eDNA detection probabilities (de Souza et al. 2016; Eichmiller et al. 2014; Jo et al. 2020; Klymus et al. 2015; but see Takahashi et al. 2018). Indeed, these behavior differences may have driven the significant differences in the intercepts of our multispecies analyses, where westslope cutthroat trout had a higher intercept than bull trout.

Site was a well-supported and significant random effect in our models, yet site-specific habitat covariates had minimal support. These contradictory results suggest that the measured habitat attributes were incomplete or inadequate for our purposes or that landscape-level characteristics could be influential. The habitat attributes evaluated in this study are commonly measured by many salmonid fish monitoring programs in the Pacific Northwest and beyond (Kershner et al. 2004), because they provide meaningful insight about critical salmonid life stages such as spawning and rearing. The hope was that pairing eDNA sampling with frequently and widely conducted salmonid habitat assessments would increase efficiency in resource use and gain greater inference over costly, site-specific abundance estimates. Several of these habitat attributes have been associated with eDNA concentrations in other studies, such as water temperature, which influences eDNA production and degradation (Eichmiller et al. 2014; Seymour et al. 2018); discharge, which influences eDNA transport (Jerde et al. 2016; Pont et al. 2018); and substrate composition, which influences eDNA retention (Shogren et al. 2017). However, these habitat variables were not associated with eDNA concentration monitoring of our three target species. Alternative environmental variables, informed by a better understanding of eDNA dynamics (production, transport, and persistence), should be considered, rather than those informed by salmonid life histories.

Pairing eDNA sampling with other field monitoring programs is a powerful way to provide easy to collect, cost-effective information about target species distribution and occurrence across broad landscapes (e.g., Wilcox et al. 2018). As others have stated, the cost of eDNA sampling and analysis is often less than the personnel cost of traveling to and traditionally sampling remote or distant sites (Pilliod et al. 2019; Sepulveda et al. 2019; Wilcox et al. 2016). Though it may be cost-effective to have habitat monitoring crews collect eDNA samples, our results indicate that paired eDNA sampling did little to improve the accuracy of eDNA concentration – fish abundance predictions at ~100-m long stream sites. At these smaller spatial scales, either eDNA sampling methods or our ability to characterize environmental conditions relevant to eDNA dynamics require further refinement. Future research should assess whether eDNA analyses provide a more tractable means of inferring fish population trends at larger spatial and longer temporal scales that more appropriately match the life histories of the target species and the scales of management actions and human land use.

Contributions

A.J.S., R.A., M.B.L., K.C., D.S.P., and A.Z. designed the study; M.B.L., B.M., and K.C. collected the field data; A.J.S., R.A., and M.B.L. conducted analyses; and A.J.S., R.A., M.B.L., D.S.P., and K.C. wrote the manuscript.

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References

Al-Chokhachy, R., Budy, P., and Conner, M. 2009. Detecting declines in the abundance of a bull trout (*Salvelinus confluentus*) population: understanding the accuracy, precision, and costs of our efforts. Can. J. Fish. Aquat. Sci. **66**(4): 649–658. doi:10.1139/F09-026.

Al-Chokhachy, R., Roper, B.B., Bowerman, T., and Budy, P. 2010. A review of bull trout habitat associations and exploratory analyses of patterns across the interior Columbia River Basin. N. Am. J. Fish. Manage. **30**(2): 464–480. doi:10.1577/M09-034.1.

Al-Chokhachy, R., Sepulveda, A.J., Ray, A.M., Thoma, D.P., and Tercek, M.T. 2017. Evaluating species-specific changes in hydrologic regimes: an iterative approach for salmonids in the Greater Yellowstone Area (U.S.A.). Rev. Fish Biol. Fish. **27**(2): 425–441. doi:10.1007/s11160-017-9472-3.

Archer, E.K., Henderson, R., Ojala, J.V., Gavin, A., and Burke, K.K. 2016. PacFish InFish Biological Opinion (PIBO) Monitoring Program: Effectiveness Monitoring Sampling Methods for Stream Channel Attributes. US Department of Agriculture, Logan, Utah.

Arterburn, J., Kistler, K., Wagner, P., Nugent, J., and Dasher, R. 2007. Okanogan Basin Monitoring and Evaluation Program Field Manual — Okanogan Basin Physical Habitat Monitoring Version 2.2.

Barnes, M.A., and Turner, C.R. 2016. The ecology of environmental DNA and implications for conservation genetics. Conserv. Genet. **17**(1): 1–17. doi:10.1007/s10592-015-0775-4.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., et al. 2015. Package 'lme4'. Convergence, **12**(1).

Bolker, B. 2014. Maximum likelihood estimation and analysis with the bbmle package. R package version 1.17.

Carim, K., Wilcox, T., Young, M., McKelvey, K., and Schwartz, M. 2015. Protocol for collecting eDNA samples from streams [Version 2.1]. US Department of Agriculture Forest Service, Missoula, Mont.

Chambert, T., Pilliod, D.S., Goldberg, C.S., Doi, H., and Takahara, T. 2018. An analytical framework for estimating aquatic species density from environmental DNA. Ecol. Evol. **8**(6): 3468–3477. doi:10.1002/ece3.3764. PMID:29607039.

Chapman, D.G. 1954. The estimation of biological populations. Ann. Math. Statist. **25**(1): 1–15. doi:10.1214/aoms/1177728844.

Cooke, S., Woodley, C., Eppard, M., Brown, R., and Nielsen, J. 2011. Advancing the surgical implantation of electronic tags in fish: A gap analysis and research agenda based on a review of trends in intracoelomic tagging effects studies. Rev. Fish Biol. Fish. **21**: 127–151. doi:10.1007/s11160-010-9193-3.

de Souza, L.S., Godwin, J.C., Renshaw, M.A., and Larson, E. 2016. Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. PLoS ONE, **11**(10): e0165273. doi:10.1371/journal.pone.0165273. PMID:27776150.

Deiner, K., Walser, J.-C., Mächler, E., and Altermatt, F. 2015. Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. Biol. Conserv. **183**: 53–63. doi:10.1016/j.biocon.2014.11.018.

Doi, H., Inui, R., Akamatsu, Y., Kanno, K., Yamanaka, H., Takahara, T., and Minamoto, T. 2017. Environmental DNA analysis for estimating the abundance and biomass of stream fish. Freshw. Biol. **62**(1): 30–39. doi:10.1111/fwb.12846.

Dysthe, J.C., Franklin, T.W., McKelvey, K.S., Young, M.K., and Schwartz, M.K. 2018. An improved environmental DNA assay for bull trout (*Salvelinus confluentus*) based on the ribosomal internal transcribed spacer I. PLoS ONE, **13**(11): e0206851. doi:10.1371/journal.pone.0206851. PMID:30399172.

Eichmiller, J.J., Bajer, P.G., and Sorensen, P.W. 2014. The relationship between the distribution of common carp and their environmental DNA in a small lake. PLoS ONE, **9**(11): e112611. doi:10.1371/journal.pone.0112611. PMID:25383965.

Fausch, K.D., Torgersen, C.E., Baxter, C.V., and Li, H.W. 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes: a continuous view of the river is needed to understand how processes interacting among scales set the context for stream fishes and their habitat. Bioscience, **52**(6): 483–498. doi:10.1641/0006-3568(2002)052[0483:LTRBTG]2.0.CO;2.

Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., et al. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol. Evol. **7**(11): 1299–1307. doi:10.1111/2041-210X.12595.

Grueber, C., Nakagawa, S., Laws, R., and Jamieson, I. 2011. Multimodel inference in ecology and evolution: challenges and solutions. J. Evol. Biol. **24**(4): 699–711. doi:10.1111/j.1420-9101.2010.02210.x. PMID:21272107.

Ham, K.D., and Pearsons, T.N. 2000. Can reduced salmonid population abundance be detected in time to limit management impacts? Can. J. Fish. Aquat. Sci. **57**(1): 17–24. doi:10.1139/f99-175.

Hillman, T.W., and Board, U.C.S.R. 2004. Monitoring strategy for the Upper Columbia basin. Prepared for: Upper Columbia Regional Technical Team, Upper Columbia Salmon Recovery Board, Wenatchee, Washington.

ISEMP–CHaMP. 2017. Integrated Status and Effectiveness Monitoring Program (ISEMP) and Columbia Habitat Monitoring Program (CHaMP) Annual Combined Technical Report. BPA Projects 2003-017-00 and 2011-006-00.

Jane, S.F., Wilcox, T.M., McKelvey, K.S., Young, M.K., Schwartz, M.K., Lowe, W.H., et al. 2015. Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. Mol. Ecol. Resourc. **15**(1): 216–227.

Jerde, C.L., Olds, B.P., Shogren, A.J., Andruszkiewicz, E.A., Mahon, A.R., Bolster, D., and Tank, J.L. 2016. Influence of stream bottom substrate on retention and transport of vertebrate environmental DNA. Environ. Sci. Technol. **50**(16): 8770–8779. doi:10.1021/acs.est.6b01761. PMID:27409250.

Jo, T., Arimoto, M., Murakami, H., Masuda, R., and Minamoto, T. 2020. Estimating shedding and decay rates of environmental nuclear DNA with relation to water temperature and biomass. Environ. DNA, **2**: 140–151. doi:10.1002/edn3.51.

Kershner, J.L., Roper, B.B., Bouwes, N., Henderson, R., and Archer, E. 2004. An analysis of stream habitat conditions in reference and managed watersheds on some federal lands within the Columbia River basin. N. Am. J. Fish. Manage. **24**(4): 1363–1375. doi:10.1577/M03-002.1.

Klymus, K.E., Richter, C.A., Chapman, D.C., and Paukert, C. 2015. Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. Biol. Conserv. **183**: 77–84. doi:10.1016/j.biocon.2014.11.020.

Klymus, K.E., Merkes, C.M., Allison, M.J., Goldberg, C.S., Helbing, C.C., Hunter, M.E., et al. 2020. Reporting the limits of detection and quantification for environmental DNA assays. Environ. DNA, **2**: 271–282. doi:10.1002/edn3.29.

Kuznetsova, A., Brockhoff, P.B., and Christensen, R.H.B. 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Soft. **82**(13): 1–26. doi:10.18637/jss.v082.i13.

Lacoursière-Roussel, A., Côté, G., Leclerc, V., and Bernatchez, L. 2016. Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. J. Appl. Ecol. **53**(4): 1148–1157. doi:10.1111/1365-2664.12598.

Laramie, M.B., Pilliod, D.S., and Goldberg, C.S. 2015*a*. Characterizing the distribution of an endangered salmonid using environmental DNA analysis. Biol. Conserv. **183**: 29–37. doi:10.1016/j.biocon.2014.11.025.

Laramie, M.B., Pilliod, D.S., Goldberg, C.S., and Strickler, K.M. 2015*b*. Environmental DNA sampling protocol — Filtering water to capture DNA from aquatic organisms. *In* US Geological Survey Techniques and Methods, Book 2. p. 15.

Levi, T., Allen, J.M., Bell, D., Joyce, J., Russell, J.R., Tallmon, D.A., et al. 2019. Environmental DNA for the enumeration and management of Pacific salmon. Mol. Ecol. Resour. **19**(3): 597–608. doi:10.1111/1755-0998.12987. PMID:30592374.

Lüdecke, D., Makowski, D., and Waggoner, P. 2019. Performance: assessment of regression models performance. R package version 0.42.

McIntyre, J.D., and Rieman, B.E. 1995. Westslope cutthroat trout. *In* Conservation assessment for inland cutthroat trout. US Forest Service General Technical Report RM-256. pp. 1–15.

Muhlfeld, C.C., and Marotz, B. 2005. Seasonal movement and habitat use by subadult bull trout in the upper Flathead River system, Montana. N. Am. J. Fish. Manage. **25**(3): 797–810. doi:10.1577/M04-045.1.

Muhlfeld, C.C., Dauwalter, D.C., Kovach, R.P., Kershner, J.L., Williams, J.E., and Epifanio, J. 2018. Trout in hot water: A call for global action. Science, **360**(6391): 866–867. doi:10.1126/science.aat8455. PMID:29798877.

Nakano, S., Kitano, S., Nakai, K., and Fausch, K.D. 1998. Competitive interactions for foraging microhabitat among introduced brook charr, *Salvelinus fontinalis*, and native bull charr, *S. confluentus*, and westslope cutthroat trout, *Oncorhynchus clarki lewisi*, in a Montana stream. Environ. Biol. Fishes. **52**(1–3): 345–355. doi:10.1023/A:1007359826470.

Nickum, J., Bart, H., Jr., Bowser, P., Greer, I., Hubbs, C., Jenkins, J., et al. 2004. Guidelines for the use of fishes in research. American Fisheries Society. 0363-2415.

OBMEP. 2017. Okanogan Basin Monitoring and Evaluation Program, 2016 Annual Report. Colville Confederated Tribes Report submitted to the Bonneville Power Administration, Project No. 2003-022-00, Nespelem, Wash.

Ogle, D.H., Wheeler, P., and Dinno, A. 2018. FSA: fisheries stock analysis. R package version 0.8.22. Available from https://github.com/droglenc/FSA.

Peterson, J.T., Thurow, R.F., and Guzevich, J.W. 2004. An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. Trans. Am. Fish. Soc. **133**(2): 462–475. doi:10.1577/03-044.

Pilliod, D.S., Goldberg, C.S., Arkle, R.S., and Waits, L.P. 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Can. J. Fish. Aquat. Sci. **70**(8): 1123–1130. doi:10.1139/cjfas-2013-0047.

Pilliod, D.S., Laramie, M.B., MacCoy, D., and Maclean, S. 2019. Integration of eDNA-based biological monitoring within the US Geological Survey's National Streamgage Network. J. Am. Water Resour. Assoc. **55**(6): 1505–1518. doi:10.1111/1752-1688.12800.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S., Van Willigen, B., and Maintainer, R. 2017. Package 'nlme'. Linear and nonlinear mixed effects models, version 3.

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., et al. 2018. Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. Sci. Rep. **8**(1): 10361. doi:10.1038/s41598-018-28424-8. PMID:29991759.

R Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Sepulveda, A.J., Schmidt, C., Amberg, J., Hutchins, P., Stratton, C., Mebane, C., et al. 2019. Adding invasive species biosurveillance to the US Geological Survey streamgage network. Ecosphere, **10**(8). e02843. doi:10.1002/ecs2.2843.

Sepulveda, A.J., Hutchins, P.R., Jackson, C., Ostberg, C., Laramie, M.B., Amberg, J., et al. 2020. A round-robin evaluation of the repeatability and reproducibility of environmental DNA assays for dreissenid mussels. Environ. DNA, **2**: 446–459. doi:10.1002/edn3.68.

Seymour, M., Durance, I., Cosby, B.J., Ransom-Jones, E., Deiner, K., Ormerod, S.J., et al. 2018. Acidity promotes degradation of multi-species

environmental DNA in lotic mesocosms. Commun. Biol. **1**: 1. doi:10.1038/s42003-017-0002-6. PMID:29809203.

Shogren, A.J., Tank, J.L., Andruszkiewicz, E., Olds, B., Mahon, A.R., Jerde, C.L., and Bolster, D. 2017. Controls on eDNA movement in streams: Transport, retention, and resuspension. Sci. Rep. **7**(1): 5065. doi:10.1038/s41598-017-05223-1. PMID:28698557.

Shogren, A.J., Tank, J.L., Egan, S.P., Bolster, D., and Riis, T. 2019. Riverine distribution of mussel environmental DNA reflects a balance among density, transport, and removal processes. Freshwater Biol. **64**(8): 1467–1479. doi:10.1111/fwb.13319.

Simpkins, D.G., and Hubert, W.A. 1996. Proposed revision of the standard-weight equation for rainbow trout. J. Freshw. Ecol. **11**(3): 319–325. doi:10.1080/02705060.1996.9664454.

Takahashi, M.K., Meyer, M.J., Mcphee, C., Gaston, J.R., Venesky, M.D., and Case, B.F. 2018. Seasonal and diel signature of eastern hellbender environmental DNA. J. Wild. Manage. **82**(1): 217–225. doi:10.1002/jwmg.21349.

The Colville Confederated Tribes, Arterburn, J., and Kistler, K. 2007. Okanogan Basin Monitoring and Evaluation Program Field Manual — Snorkel Survey Methodology Final Draft.

The Colville Confederated Tribes, Arterburn, J., Kistler, K., and Rayton, M. 2005. Okanogan Basin Monitoring and Evaluation Program Field Manual — Water Quality Sampling Protocols.

Tillotson, M.D., Kelly, R.P., Duda, J.J., Hoy, M., Kralj, J., and Quinn, T.P. 2018. Concentrations of environmental DNA (eDNA) reflect spawning salmon abundance at fine spatial and temporal scales. Biol. Conserv. **220**: 1–11. doi:10.1016/j.biocon.2018.01.030.

Wilcox, T.M., Carim, K.J., McKelvey, K.S., Young, M.K., and Schwartz, M.K. 2015. The dual challenges of generality and specificity when developing environmental DNA markers for species and subspecies of *Oncorhynchus*. PLoS ONE, **10**(11): e0142008. doi:10.1371/journal.pone.0142008. PMID:26536367.

Wilcox, T.M., McKelvey, K.S., Young, M.K., Sepulveda, A.J., Shepard, B.B., Jane, S.F., et al. 2016. Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. Biol. Conserv. **194**: 209–216. doi:10.1016/j.biocon.2015.12.023.

Wilcox, T.M., Young, M.K., McKelvey, K.S., Isaak, D.J., Horan, D.L., and Schwartz, M.K. 2018. Fine-scale environmental DNA sampling reveals climate-mediated interactions between native and invasive trout species. Ecosphere, **9**(11): e02500. doi:10.1002/ecs2.2500.

Yates, M.C., Fraser, D.J., and Derry, A.M. 2019. Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. Environ. DNA, **1**: 5–13. doi:10.1002/edn3.7.

Zuur, A., Leno, E.N., Walker, N., Saveliev, A.A., and Smith, G.M. 2009. Mixed effects models and extensions in ecology with R. Springer Science & Business Media.