Identifying distribution boundaries at the upper extent of fish in streams using environmental DNA

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Introduction

Delimiting geographic distribution boundaries of species is fundamental for conservation and management decision-making. Forest management in the Pacific Northwest occurs across the landscape, including at or near the upstream extent of fish distributions in headwater streams. Stream reaches with fish have more regulatory protections and wider riparian buffers than fishless reaches (Blinn and Kilgore 2001; Lee, Smyth, and Boutin 2004). Consequently, this nexus has become the focus of questions for contemporary forest practices and fisheries.

Although there are multiple approaches that are accepted under Forest Practices Rules for Washington to delimit the last-fish, electrofishing is currently the most widely used method because it allows for detection in real time (WA DNR 2002). However, electrofishing can be time-consuming, labor-intensive (Evans et al. 2017), can harm fishes, and is a poor tool for detecting fish in low abundances (Peterson et al. 2004; Rosenberger and Dunham 2005). Alternatively, environmental DNA (eDNA) is a rapidly evolving state-of-the-art method that measures target DNA that is left behind in water and consequently does not harm fishes (Goldberg et al. 2015; Wilcox et al. 2016). eDNA has been shown to be comparable to, or more sensitive at, detecting fish than electrofishing in streams (Wilcox et al. 2016; Baldigo et al. 2017; Evans et al. 2017; Ostberg et al. 2019), particularly when species are low in abundance (Dejean et al. 2012; Pilliod et al. 2013; Sigsgaard et al. 2015; Itakura et al. 2019). Despite the expansion of eDNA approaches into monitoring and inventory programs around the globe, issues remain with detections of false positives and false negatives (Roussel et al. 2015, Guillera-Arroita et al. 2017). These management-relevant approaches have yet to be evaluated to understand their abilities to detect the upper extent of fish in streams.

Here, we evaluate the relative reliability of eDNA of Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii)* as a management tool to detect the upper extent of fish. To do so, we compare the upper fish distribution from eDNA to standard electrofishing for a small number (n=12) of forested streams in Washington. Coastal Cutthroat Trout are the fish generally found the highest up in their stream network across their range (Budy et al. 2019). All sampling coincided with the recognized defined sampling window for evaluating the upper extent of fish under Forest Practices Rules in Washington (March 1st to July 15th). We (1) identify whether eDNA can be used as a management tool to identify the upper boundary of fish by evaluating whether it detects fish at the same sites as electrofishing within each stream and/or above the boundary identified by electrofishing; and (2) identify operational limitations to using eDNA for determining the last-fish. In addition, because the sampling of these 12 streams occurred within a broader study across Washington and Oregon, we also (3) provide estimates of fish detection probabilities of eDNA across a broader suite of sites (Penaluna et al. *in press*). We predict that eDNA will detect fish further upstream than electrofishing across streams because of its acknowledged strength for identifying species in low abundance, as is often the case for fishes near their upper distribution boundary. Ultimately, our results provide a comparison of a

standard field method and a rapidly advancing technique for examining presence of fish in small streams.

Methods

Study streams and sample design

We sampled 12 streams in collaboration with Hancock Forest Management, Port Blakely, and Weyerhaeuser Company on their land (Fig. 1). We worked with landowners to select streams by prioritizing streams with previous information related to the upper extent of Coastal Cutthroat Trout (*O. clarkii clarkii*). Our sampling framework relied on prior documentation of the upstream extent of fish presence identified through a previous fish distribution survey, and, consequently, we initiated sampling at least 175m downstream of these previous boundaries.

Environmental DNA samples were collected on the same day as electrofishing, but immediately in advance of electrofishing to decrease contamination risk for eDNA and compare approaches. We collected eDNA from eight discrete sampling sites located every 50 m moving upstream (Fig. 2). Generally three sites were downstream of the last–fish, which was determined at the time of electrofishing, and the remaining sites were upstream, except for streams C, D, E, F, and I. To ensure eDNA sampling locations met the targeted sampling design (i.e., located above and below of the last fish location as identified by electrofishing), additional eDNA samples (>8) were often collected with subsequent processing limited to the 8 locations that met the study design criteria. Sample spacing of 50m was selected to offer additional point information on the detection probabilities of fish above and below where fish were noted during continuous electrofishing. Consequently, the last-fish observed by electrofishing often occurred between sites 3 and 4 with about 100 m downstream of that point and 250 m above.

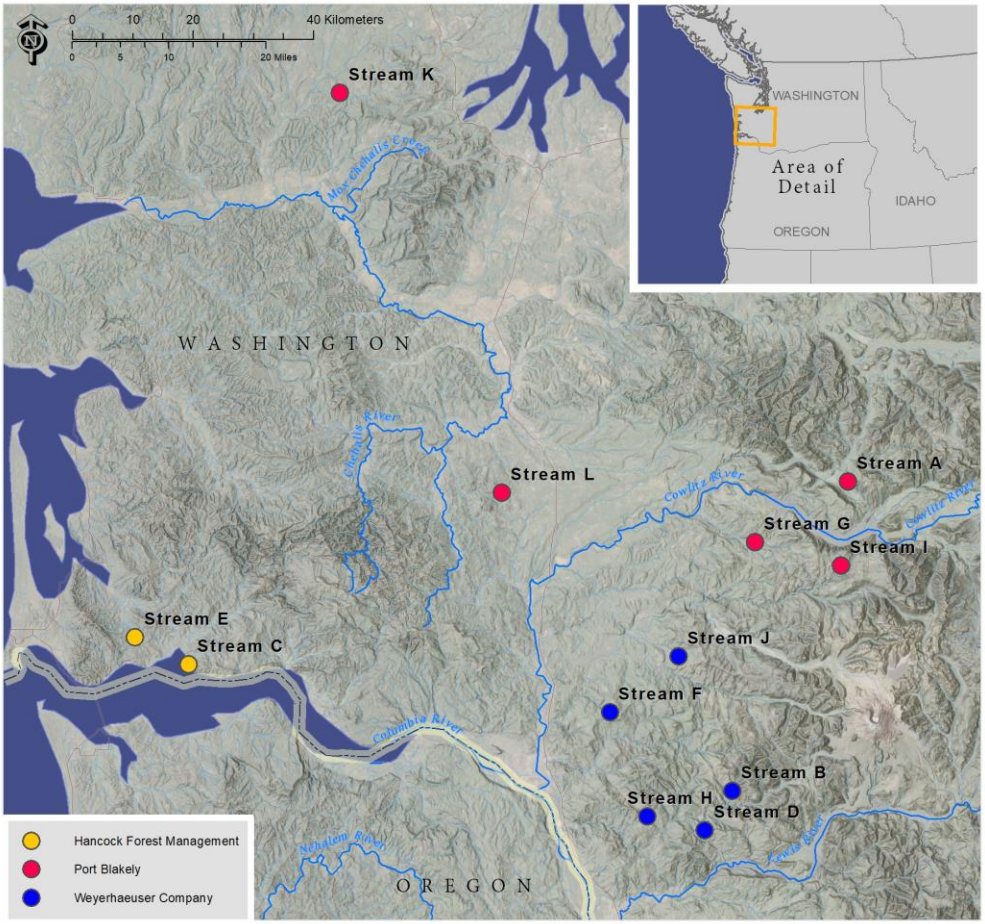

Figure 1. Map of twelve study streams in Southwest Washington. At each stream, the upper extent of fish was evaluated with electrofishing and eDNA.

eDNA sampling

At each stream, we collected 1L water samples in triplicate from the thalweg at the downstream end of each 50 m electrofishing sampling unit for each of the 8 sites. Samples were collected in triplicate to account for imperfect detection of eDNA (Hunter et al. 2015). We pumped sample water through 0.45 micron single–use cellulose nitrate filters (Sterlitech, Kent, WA) using a vacuum pump. Water was collected with either a 1L Nalgene bottle or a 1L disposable sterile Whirlpak bag and held in the stream to remain cool for 1–3 hours while other samples were collected from each site. Samples were picked up and sorted based on last–fish observed by electrofishing. Filters were loosely rolled and stored frozen in 5mL vials on wet ice during transport, and were frozen at –20°C within 6 hours of collection. Filters were stored at –20°C until DNA extraction. Bottles and tweezers were sterilized with a 50% bleach solution followed by a triple deionized water rinse before use.

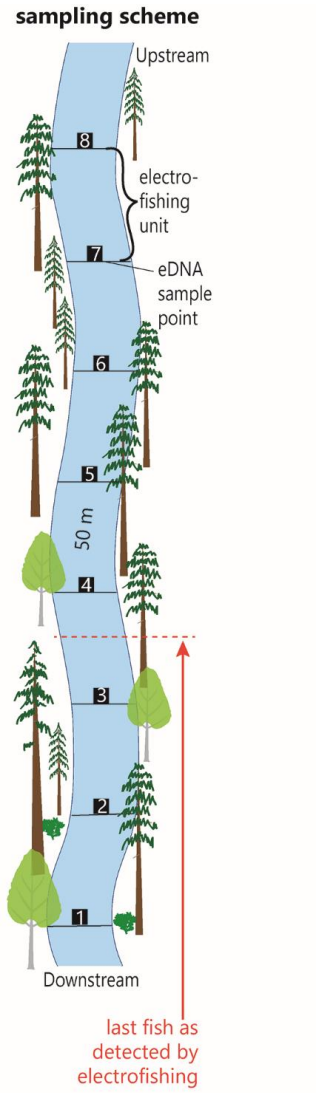

Figure 2. Schematic of eDNA sampling collection, including 8 eDNA sampling points at the downstream end of each electrofishing unit. Generally, the last-fish detected by electrofishing fell between sites 3 and 4, except for streams C, D, E, F, and I.

DNA was extracted from each filter using a modified protocol of the Qiagen DNeasy Blood and Tissue kit (Levi et al. 2018). Specifically, we added 1.0 mm zirconia–silica beads to the initial lysis buffer followed by a 15-minute vortex step to loosen the DNA from the filters. Incubation in lysis buffer was increased to 48 hours. After incubation, 300ul of the lysed product was transferred to a new 1.7ml microcentrifuge tube. Thereafter, we followed the manufacturer's protocol for isolation of tissue. DNA was eluted in a total volume of 100ul. All DNA extractions and PCR setup are done inside of separate hepa–filtered and UV–irradiated PCR cabinet (Air Science LLC, Fort Meyers, FL) in a separate lab where no PCR products or other sources of high concentration DNA are allowed.

There are currently no consistent criteria for determining what is considered a positive detection for eDNA (Goldberg et al. 2016). We consider detection of trout DNA in a sample as a positive signal from a single replicate out of 9 possible replicates (3 field replicates x 3 qPCR or

technical replicates), but also recognize that a single positive sample provides weak evidence of species presence relative to consistent positive samples across replicates over time (Jerde et al. 2011).

eDNA quantitative PCR

We used a species–specific assay for Coastal Cutthroat Trout that targets the cytochrome oxidase I of the mitochondrial genome for trout in the study area. Each sample was run in triplicate PCR reactions. PCR was performed using quantitative PCR (qPCR; Biorad). Each 20ul qPCR reaction contained 6ul of DNA template, 10ul Environmental Master Mix 2.0 (ThermoFisher Scientific, Waltham, MA), 0.2 uM of both forward and reverse primers, 0.2um of the TaqMan MGB probe, and sterile water. Additionally, each plate contained a four-point standard curve using DNA obtained from Coastal Cutthroat Trout tissue. Extracted tissue was quantified using a Qubit Fluorometer (ThermoFisher Scientific, Waltham, MA) and diluted from 10^{-1} ng/ul to 10^{-4} ng/ul. PCR cycling conditions involved an initial denaturation step of 10 min at 95C to activate the HotStart Taq DNA polymerase, followed by 50 cycles of 95C for 15 s and 60C for 60 s. All reaction plates contained a negative control of water and extraction blanks.

Electrofishing sampling and physical habitat surveys

After eDNA water samples were collected at the downstream sampling point in each stream, we sampled the entire 50 m electrofishing unit using standard backpack electrofishing to determine the end-of-fish. We used a spatially continuous, single–pass backpack electrofishing approach similar to that described by Torgerson et al. (2004) and validated by Bateman et al. (2005). but sampling all accessible habitats We electrofished to compare relative abundance data between both approaches, and consequently, our protocol differs from typical electrofishing to identify the upper extent of fish where fish are not typically netted (WA DNR 2002). Electrofishing settings were set to the appropriate settings for each stream. We measured total length (mm) and weight (g; to tenths) of each fish captured. We processed fish, at least, at each 50-m reach break for a total of 350m of electrofishing per stream.

Physical stream habitat surveys were conducted for each 50-m reach, including channel unit type (pool, riffle, cascade), channel unit length (m), depth (m), wetted-width (m), and bankfull-width (m) at the lateral and longitudinal mid-point, gradient (recorded to nearest whole number (%), and dominant substrate classification (boulder, cobble, bedrock).

133 **Table 1.** Stream characteristics for 12 study streams.

Ownership	Location	Stream	Latitude last-fish[1]	Longitude last-fish[1]	Electrofishing presence	eDNA presence	Last fish higher in stream with which method?
Port Blakely	Coast	Stream L	NA	NA	No fish detected	1,3	eDNA
Port Blakely	Coast	Stream K	NA	NA	No fish detected	1,2,3,4,5,6,7,8	eDNA
Weyerhaeuser	Cascades	Stream H	46.055817	-122.681817	1,2,3	1,2,3,6,7,8	eDNA
Weyerhaeuser	Cascades	Stream F	46.211533	-122.761283	1,2,3,4	1,2,3,4,6,8	eDNA
Port Blakely	Cascades	Stream I	46.428613	-122.260414	1	1,2,6	eDNA
Port Blakely	Cascades	Stream G	46.464469	-122.446467	1,2,3	1,2,3,4,8	eDNA
Weyerhaeuser	Cascades	Stream D	46.034383	-122.558367	1,2,3,5	1,2,3,4	electrofishing
Hancock	Coast	Stream E	46.321234	-123.785673	1,2,5	1,2	electrofishing
Weyerhaeuser	Cascades	Stream B	46.093533	-122.4993	1,2,3	1,2,3	same last-fish boundary
Weyerhaeuser	Cascades	Stream J	46.294667	-122.612517	1,2,3	1,2,3,4	same last-fish boundary
Port Blakely	Cascades	Stream A	46.553818	-122.243428	1,2,3	1,2,3	same last-fish boundary
Hancock	Coast	Stream C	46.281334	-123.669986	1,2	1,2	same last-fish boundary

134 [1]determined by electrofishing

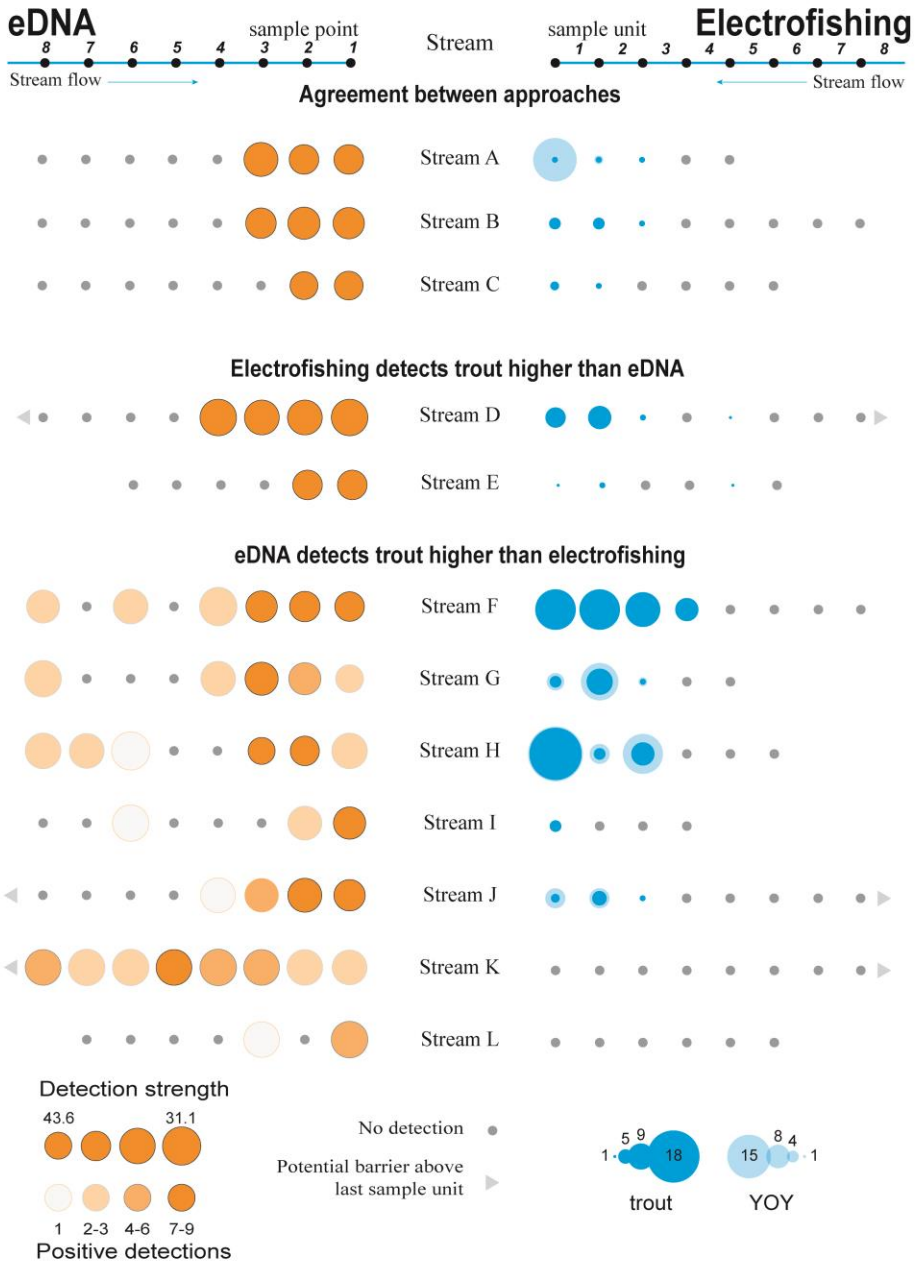

Figure 3. Patterns of agreement and disagreement in Coastal Cutthroat Trout detection between methods using eDNA relative abundances and electrofishing over 8 sites across twelve sample streams on Weyerhaeuser Company, Port Blakely, and Hancock Forest Management land in Washington. In the upper section, we illustrate streams where both methods, eDNA (orange) and electrofishing (blue), showed full agreement as to the upper extent of fish. Gray circles represent no detection and gray x's represent not sampled for both methods. In the middle section, we illustrate streams where electrofishing detected trout upstream of eDNA. Size of the symbols represents eDNA detection strength (threshold cycle value: Cq) and fish abundance from electrofishing (#/per 50 m sample unit). The lower section shows streams where eDNA detected trout above electrofishing. Each row represents a single stream with arrows indicating stream

flow direction (eDNA is from left to center mirroring electrofishing, which is from right to center). Dark orange shows higher detection amongst eDNA replicates, whereas light orange is the opposite. Dark blue shows captures of adult Coastal Cutthroat Trout (trout) and light blue shows captures of young-of-year (YOY), which could either be *O. mykiss* or *O. clarkii clarkii*.

Data Analysis

We compared the proportion of agreement between the detection of trout by eDNA and electrofishing across streams and sites. We displayed information for all results across streams and sites, including all field and qPCR replicates, to reveal the variability in eDNA results, especially because we are near the lower detection limits of the focal species at the upper extent of their distribution.

Occupancy Modeling Approach using broader suite of sites in Washington and Oregon

Because eDNA is heterogeneously distributed in water, there is imperfect detection and to account for this imperfect detection, we used occupancy models to estimate detection probabilities (Hunter et al. 2015). For the following analyses, we used the results from the 12 CMER streams in addition to 19 streams in both Oregon and Washington that followed the same protocol for a more robust analyses that improves the confidence of the modeling.

We used a three-level occupancy model EDNAOCCUPANCY in R that uses Bayesian methods of analysis of Markov Chain Monte Carlo (MCMC) methods of maximum-likelihood to estimate model parameters [i.e., $\Psi(.)\theta(.)p(.)$] and include covariates (Dorazio and Erickson 2017). Accordingly, we can estimate fish detection probabilities while also estimating the conditional probability of detecting trout DNA that may be present in a field sample or qPCR replicate. The three levels of sampling included aspects of the nested sampling design innate in eDNA sampling of location (stream x site), field sample, and qPCR replicate. In the model, Ψ is the probability that the eDNA is present at a location, θ is the conditional probability that eDNA occurred in a replicate sample given that it occurred at the location level, and p is the conditional probability of detecting eDNA in a replicate qPCR reaction given that it occurred at the field sample level.

We predicted that Ψ might vary across stream locations due to physical characteristics of the stream sections. However, after initial data analyses, wetted width and depth were the only characteristics that influenced the results and remained in the model. Accordingly, we evaluated how Ψ might vary across the size of streams locations, including stream width and depth. In addition, θ and p might be influenced by the abundances of trout or all fishes detected by electrofishing due to eDNA inhibition or molecular competition in qPCR reactions. We evaluated several models that included a different combination of covariates at different scales (Ψ, θ, and/or p). Covariates were measured at the location-level including single-pass standard electrofishing surveys that evaluated density of all fishes (#/50 linear m), and density of Coastal Cutthroat Trout (#/50 linear m). Covariates encompassing habitat size included stream width (m) and stream depth (m). We fitted and evaluated eight candidate models using available functions for model-selection criteria from the EDNAOCCUPANCY package. Model-selection criteria included the posterior-predictive loss criterion (PPLC) and widely applicable information criterion (WAIC). We fitted each candidate model by running the MCMC algorithm for 11,000 iterations and retaining the last 10,000 for estimating posterior summaries. After selecting the model with the greatest amount of support (lowest WAIC value and higher PPLC), we explored the estimated relationships among covariates (i.e., stream width, stream depth, trout density, and density of all captured fishes) and estimated model parameters Ψ and θ.

Lastly, we used results from the model $[\Psi(.)\theta(.)p(.)]$ that included covariates and adopted the approach described in Hunter et al. (2015) to compute the cumulative probability of detecting Coastal Cutthroat Trout eDNA in K qPCR replicated sample (p^*), given that the sample contained eDNA the model as $p^* = 1-(1-p)K$. This procedure allowed us to asses if we used an

196 adequate number of qPCR replicates to detect trout eDNA. We performed a similar analysis to
197 estimate the cumulative probability of occurrence of Coastal Cutthroat Trout eDNA in n water
198 samples (θ^*) collected from a location that contained eDNA using $\theta^* = 1-(1-\theta)n$.

Table 2. Parameter estimates (posterior mean ± SE) and model-selection criteria (PPLC and WAIC) for each candidate model of Coastal Cutthroat Trout eDNA detections for 31 streams using same protocol. Streams included 12 CMER-funded streams and 19 non-CMER funded streams. Values represent either probabilities or estimates of the coefficients of the relationship between the covariate(s) and detection probability of the form logit(Ψ) = α_0 + α_1* covariate + α_2* covariate$_2$, or logit(θ) = β_0 + β_1* covariate + β_2* covariate$_2$, or logit(P) = δ_0 + δ_1* covariate.

	Occupancy in location (Ψ)	Occupancy in sample (θ)	Detection in replicate (P)	PPLC	WAIC
Ψ(.), θ(.), P(.)	0.53 (0.46, 0.59)	0.78 (0.73, 0.83)	0.89 (0.86, 0.91)	190.176	0.3673
Ψ(depth+width), θ(trout), P(all fishes)	α_0 = 0.469 (±0.013)	β_0 = 0.930 (±0.004)	δ_0 = 1.102 (±0.001)	222.008	0.4153
	α_1 = -0.153 (±0.006)	β_1 = 2.331 (±0.025)	δ_1 = 0.191 (±0.001)		
	α_2 = 0.593 (±0.012)				
Ψ(depth), θ(trout+all fishes), P(all fishes)	α_0 = 0.649 (±0.016)	β_0 = 1.031 (±0.006)	δ_0 = 1.101 (±0.001)	222.756	0.4158
	α_1 = 0.092 (±0.004)	β_1 = 1.986 (±0.028)	δ_1 = 0.191 (±0.001)		
		β_2 = 1.863 (±0.017)			
Ψ(width), θ(trout+all fishes), P(all fishes)	α_0 = 0.635 (±0.011)	β_0 = 1.046 (±0.006)	δ_0 = 1.102 (±0.001)	222.320	0.4158
	α_1 = 0.504 (±0.004)	β_1 = 1.892 (±0.021)	δ_1 = 0.191 (±0.001)		
		β_2 = 1.918 (±0.018)			
Ψ(width), θ(trout), P(all fishes)	α_0 = 0.359 (±0.007)	β_0 = 0.928 (±0.004)	δ_0 = 1.104 (±0.001)	221.471	0.4167
	α_1 = 0.410 (±0.004)	β_1 = 2.169 (±0.022)	δ_1 = 0.192 (±0.001)		
Ψ(.), θ(trout), P(all fishes)	0.63 (0.51, 0.79)	β_0 = 0.938 (±0.004)	δ_0 = 1.104 (±0.001)	221.540	0.4172
		β_1 = 2.231 (±0.029)	δ_1 = 0.194 (±0.001)		
Ψ(width+depth), θ(trout+all fishes), P(all fishes)	α_0 = 0.801 (±0.011)	β_0 = 1.034 (±0.006)	δ_0 = 1.102 (±0.001)	222.940	0.4174
	α_1 = 0.851 (±0.010)	β_1 = 1.936 (±0.017)	δ_1 = 0.189 (±0.001)		
	α_2 = -0.267 (±0.006)	β_2 = 1.971 (±0.019)			
Ψ(depth), θ(trout), P(all fishes)	α_0 = 0.329 (±0.008)	β_0 = 0.936 (±0.004)	δ_0 = 1.104 (±0.001)	221.562	0.4181
	α_1 = 0.122 (±0.002)	β_1 = 2.184 (±0.026)	δ_1 = 0.193 (±0.001)		

Results

Three streams (25%) agreed to the upper extent of fish for both approaches (Table 1; Fig. 3). Trout eDNA was detected above the last observed fish with electrofishing in seven streams (58%) by 50–250 m. Two of these seven streams did not have any trout observed with electrofishing (Streams K and L). The most upstream trout detections with eDNA had fewer replicate eDNA detections than downstream sites in the same stream, often 1 to 3 of 9 total replicates. Two other streams (17%) resulted in fish observed 50–150 m higher with electrofishing than eDNA. All study streams had trout detections with eDNA.

Model results using broader suite of sites in Washington and Oregon

The model with covariates that had the greatest support revealed that (i) stream widths positively correlate with eDNA trout occupancy at location; (ii) electrofishing trout densities positively affect eDNA field samples; (iii) and electrofishing densities of all fish positively influence the quantity of positive qPCR replicates. Modeled results revealed that the predicted occurrence of trout eDNA was higher in wider stream locations (Table 2; Fig. 4). The occurrence of trout eDNA is increased in field samples with greater electrofishing trout density. eDNA detected trout at very low electrofishing densities of <5 trout per 50 lineal m. The occurrence of trout eDNA is greater in qPCR replicates with greater electrofishing fish density.

Estimates of detection probabilities of trout eDNA (P) suggested that qPCR was effective in detecting eDNA presence in a field sample (Model [(Ψ(.), θ(.), P(.)] in Table 2). The mean estimated detection probability collected by location was 0.89 (0.86, 0.91) and consequently the cumulative probability of detecting trout eDNA (P*) was very high ranging from 0.997 to 0.999. This suggests that three qPCR replicates per eDNA sample were sufficient to detect trout eDNA when it was present in a field sample. The cumulative probability of detecting trout eDNA (θ*) resulted very high ranging from 0.980 to 0.995. This also suggests that the three eDNA samples collected was sufficient to include trout eDNA when the eDNA was present at that location.

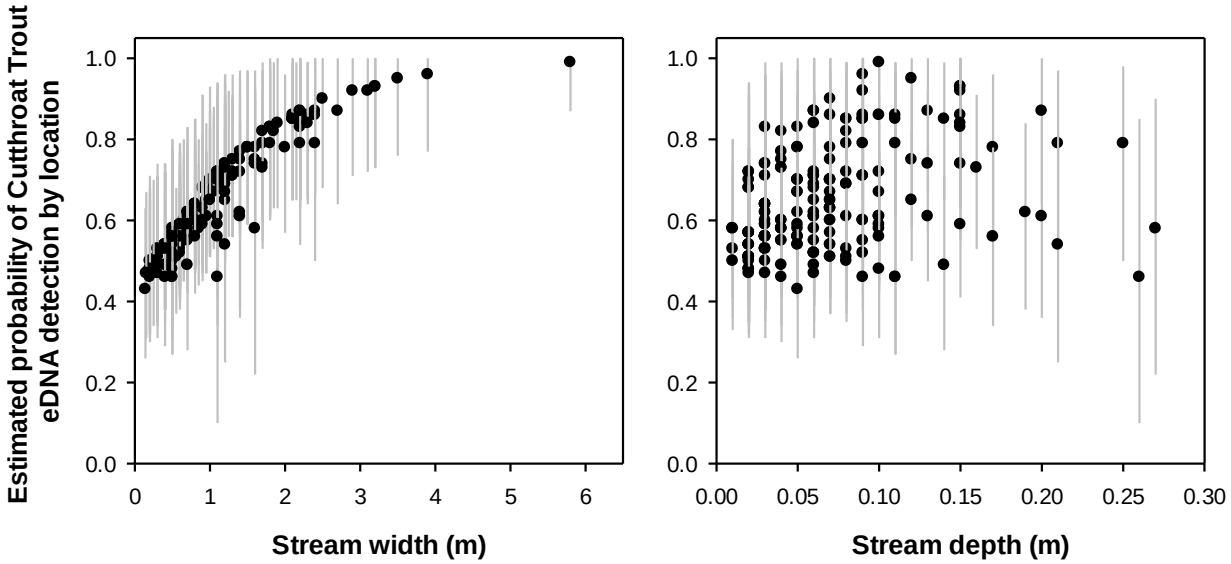

Figure 4. Estimated probabilities of occurrence of trout eDNA by location (Ψ) increases with stream width. Location is stream x site. Symbols are estimates of posterior means with 95% credible intervals for the model [Ψ(depth+width), θ(trout), P(all fishes)] described in Table 2.

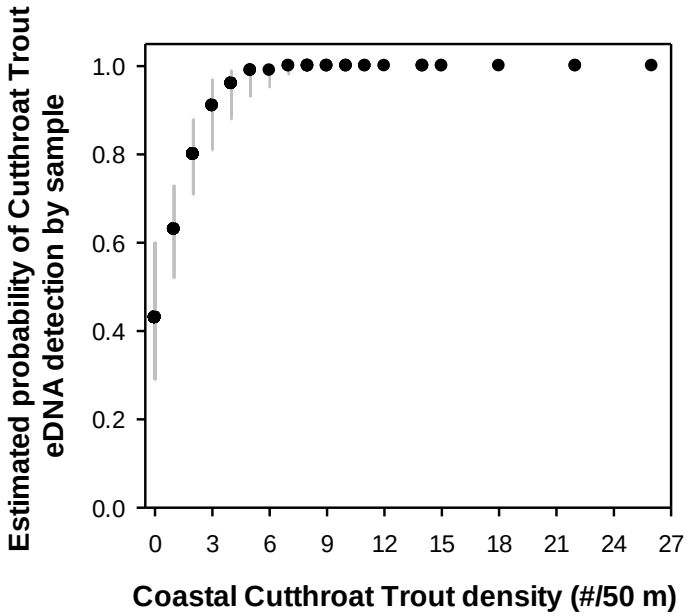

Figure 5. Estimated probabilities of occurrence of trout eDNA in field samples (θ) increases with increased trout density from electrofishing. Symbols are estimates of posterior means with 95% credible intervals of the model [Ψ(depth+width), θ(trout), P(all fishes)] described in Table 2. Field samples are 1L biological replicates that were taken in triplicate.

Discussion

Can eDNA be used to identify the upper boundary of fish?

We provide evidence that eDNA constitutes an effective addition to approaches that should be considered to identify the upper extent of fish. While the last–fish boundary matches between approaches in a quarter of the streams, in over half of the streams trout DNA is detected further upstream with eDNA than trout have been detected with electrofishing. For streams with positive DNA detections of trout, the uppermost sites generally revealed a reduced detection signal relative to downstream sites from the same stream probably from a low concentration of target DNA upstream from fewer fishes being found at the uppermost edge of fish, or from false positives. We find that eDNA detects trout DNA when they occur in extremely low quantities, but its detection ability is imperfect and so it also misses detecting trout in low quantities in some circumstances (Streams D and E). For example, it is not always clear how to translate positive eDNA detections into actual living trout (or eggs) in the stream versus detection failure or true absence (e.g., Darling and Mahon 2011, Jerde et al. 2011, Wilson et al. 2014).

Can eDNA be used in addition to electrofishing to determine the end–of–fish?

The upper boundary of fish has the same boundary between both approaches for 25% of sampled streams, and eDNA detects the boundary higher in more than half of all sampled streams suggesting that it is more sensitive than electrofishing. Both streams where electrofishing detects trout above eDNA have one trout at their upper-most fish site potentially because that one fish was below the detection limits for eDNA and/or was disturbed or moved upstream by the eDNA

crew walked upstream first. Although eDNA is equal to or more sensitive than electrofishing, it seems that using eDNA to define the upper extent of fish is near its detection limits. In most cases, it is able to detect trout in low densities, but sometimes it also misses them. Electrofishing has been the primary approach to identify the last-fish in streams for decades, but it appears that its ability to detect fish at the upper extent of fish is generally equal to or less effective than eDNA in these study streams. The lack of block net use while electrofishing may have pushed some fish into upstream habitats as they fled, such as streams D and E. Electrofishing protocols to determine last-fish do not typically use block nets, which ensure fish do not flee to adjacent habitats (Peterson et al. 2005). Block nets are used to ensure fish do not flee to adjacent habitats, however they are not typically used in electrofishing protocols to determine last-fish. At its optimal, standard backpack electrofishing is most efficient for larger fish in shallower water with ideal stream habitat conditions for conductivity, water temperature (<18°C), water transparency (good visibility), and habitat characteristics (Price and Peterson 2010) . Trout have higher capture probabilities than other fishes, such as those with coarse scales (cyprinids) or without swim bladders (sculpins). Electrofishing offers data of catch in real-time and consequently identifies the exact time and place that a fish was captured (Table 3). A main advantage of electrofishing by an experienced crew is that they have the ability to detect many fish species (although not equally across species or sizes), whereas eDNA detects only targeted fishes.

Table 3. Comparison of eDNA versus electrofishing approaches to delimit upper extent of fish. **Bold face** denotes positive characteristics of method.

Metric	eDNA	Electrofishing
Assesses potential presence and absence of fish	**Yes**	**Yes**
Estimates relative abundance of fish	**Yes**	**Yes**
Archives fish as museum voucher	No	**Yes**
Obtains data on length, weight, or fish characteristics	No	**Yes**
Obtains genetic data*	**Yes**	**Yes**
Allows for sampling year-round	with safe access	in wadeable waters
Can directly harm fish	**No**	Yes
Need state/federal scientific take permit	**No**	Yes
Offers data instantaneously	No	**Yes**
Identifies exact time and place of fish	No	**Yes**
Potential for false positives**	Yes	**No**
Potential for false negatives	Yes	Yes

*genetic data can be obtained from eDNA samples if they are sequenced in addition to standard qPCR analysis; **Electrofishing could have false positives if there are issues with field identification of target species

What are the operational limitations to the use of eDNA to determine the end–of–fish?

eDNA warrants inclusion amongst the sampling approaches considered to identify the upper extent of fish. We need to continue to push the boundaries of eDNA detections to identify where

the low eDNA detections for trout marks a distribution extension for the upper extent of Coastal Cutthroat Trout (actual presence) versus where it does not reflect an actual fish in the stream (detection failure). The effectiveness of eDNA depends on investigators being informed of the potential location of last-fish to know where to start sampling with its utility potentially being maximized when used as a complementary approach to standard methods. eDNA in streams detects DNA of the target species from flowing water that are generally located upstream of the sampling location, but the upstream distance DNA has travelled remains unknown, but is likely variable by stream and flow conditions, whereas electrofishing can identify fishes in a specific habitat type, such as a pool or riffle (Table 2). It is important to target all potential fishes with eDNA to ensure that last-fish is detected with eDNA, which may mean using multiple primer/probe sets. Although the precise time when DNA was shed into the environment by a focal organism is not known with eDNA, it has been able to show abundances of run timing of salmon (Levi et al. 2018), suggesting that sample timing needs to correspond to when fish are present. After sampling, eDNA samples still have to be extracted and analyzed leading to a time lag for results.

As managers start to incorporate eDNA surveys to detect last-fish, they may want to use more than one criterion to define a positive eDNA detection as part of a decision-making framework. For example, a threshold of a positive eDNA detection could be set for a given number of replicates to separate a consistent series of strong detections from a few weak detections, as well as incorporating information about potential barriers to fish movement and other habitat characteristics (e.g., wetlands, habitat complexity). We suggest that as the discussion of eDNA as a management tool continues it is important to distinguish between the science of eDNA (e.g., methodological sensitivities, limitations) and the implications that are derived from its information (e.g., fish presence). Although issues remain in the field of eDNA with detections of both false-positive and false-negative errors (Roussel et al. 2015, Guillera-Arroita et al. 2017), understanding such errors associated with using eDNA to delimit the last-fish will help to define more robust monitoring and management outcomes.

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