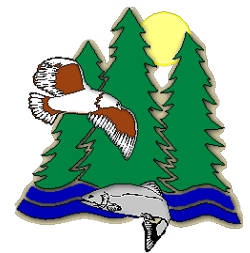


# Identifying distribution boundaries at the upper extent of fish in streams using environmental DNA

By:  
Brooke Penaluna



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## **Washington State Forest Practices Adaptive Management Program**

The Washington State Forest Practices Board (FPB) has established an Adaptive Management Program (AMP) by rule in accordance with the Forests & Fish Report (FFR) and subsequent legislation. The purpose of this program is to:

*Provide science-based recommendations and technical information to assist the FPB in determining if and when it is necessary or advisable to adjust rules and guidance for aquatic resources to achieve resource goals and objectives. The board may also use this program to adjust other rules and guidance. (Forest Practices Rules, WAC 222-12-045(1)).*

To provide the science needed to support adaptive management, the FPB established the Cooperative Monitoring, Evaluation and Research (CMER) committee as a participant in the program. The FPB empowered CMER to conduct research, effectiveness monitoring, and validation monitoring in accordance with WAC 222-12-045 and Board Manual Section 22.

### **Report Type and Disclaimer**

This project development report was prepared for the Cooperative Monitoring, Evaluation and Research Committee (CMER), and was intended to support design and implementation of Forest and Fish Adaptive Management research and monitoring studies. The project is part of the Stream Typing Program, and was conducted under the oversight of the Instream Science Advisory Group.

This document was reviewed by CMER but was not assessed through the Adaptive Management Program's independent scientific peer review process. CMER has approved this document for distribution as an official CMER document. As a CMER document, CMER is in consensus on the scientific merit of the document. However, any conclusions, interpretations, or recommendations contained within this document are those of the authors and may not reflect the views of all CMER members.

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### **Proprietary Statement**

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

Prepared by Brooke Penaluna, Lead PI (Pacific Northwest Research Station, USFS)

## 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 1<sup>st</sup> to July 15<sup>th</sup>). 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

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

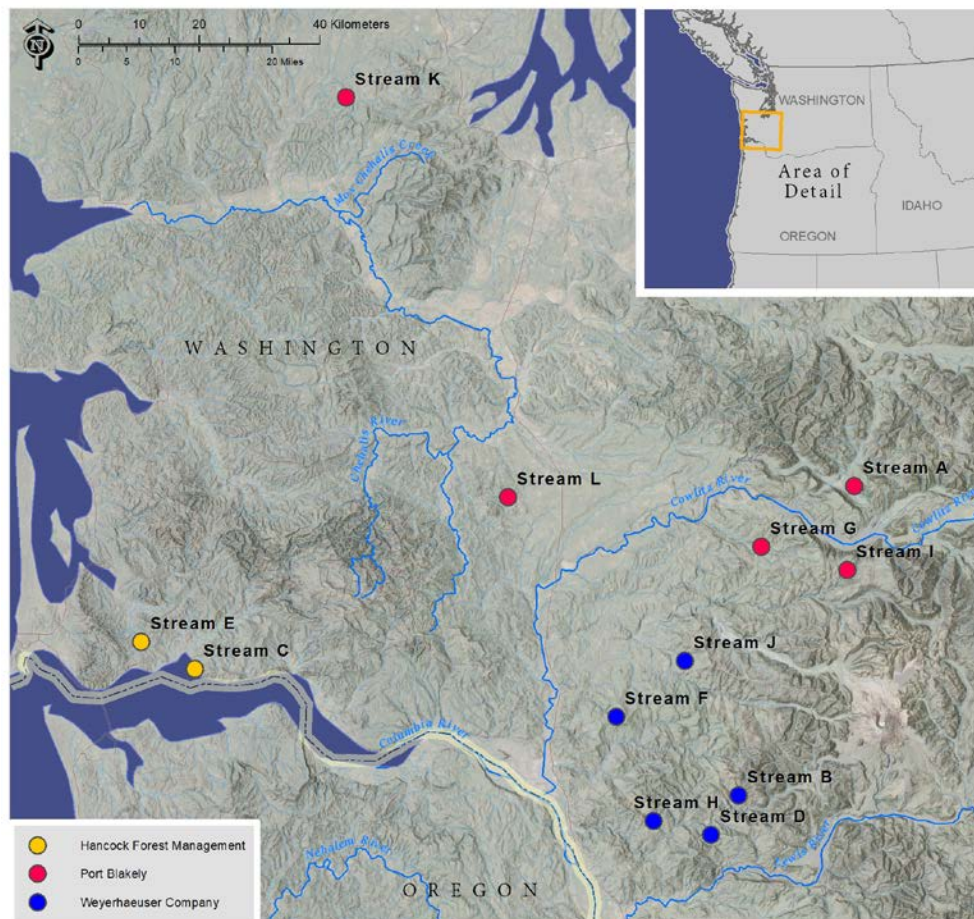
45  
46

## 47 **Methods**

### 48 *Study streams and sample design*

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

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

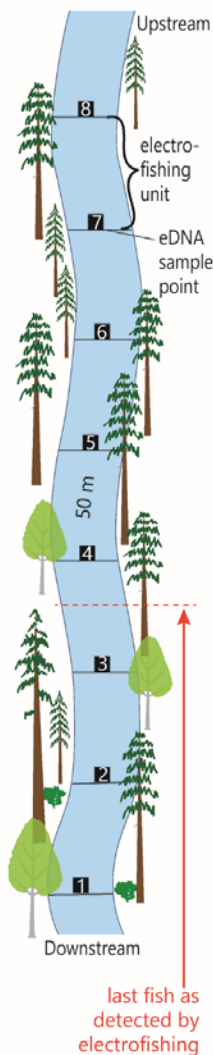


67  
68 **Figure 1.** Map of twelve study streams in Southwest Washington. At each stream, the upper  
69 extent of fish was evaluated with electrofishing and eDNA.  
70

71 *eDNA sampling*

72 At each stream, we collected 1L water samples in triplicate from the thalweg at the downstream  
73 end of each 50 m electrofishing sampling unit for each of the 8 sites. Samples were collected in  
74 triplicate to account for imperfect detection of eDNA (Hunter et al. 2015). We pumped sample  
75 water through 0.45 micron single-use cellulose nitrate filters (Sterlitech, Kent, WA) using a  
76 vacuum pump. Water was collected with either a 1L Nalgene bottle or a 1L disposable sterile  
77 Whirlpak bag and held in the stream to remain cool for 1–3 hours while other samples were  
78 collected from each site. Samples were picked up and sorted based on last-fish observed by  
79 electrofishing. Filters were loosely rolled and stored frozen in 5mL vials on wet ice during  
80 transport, and were frozen at  $-20^{\circ}\text{C}$  within 6 hours of collection. Filters were stored at  $-20^{\circ}\text{C}$   
81 until DNA extraction. Bottles and tweezers were sterilized with a 50% bleach solution followed  
82 by a triple deionized water rinse before use.  
83

### sampling scheme



84  
85 **Figure 2.** Schematic of eDNA sampling collection, including 8 eDNA sampling points at the  
86 downstream end of each electrofishing unit. Generally, the last-fish detected by electrofishing  
87 fell between sites 3 and 4, except for streams C, D, E, F, and I.  
88

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

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



101 technical replicates), but also recognize that a single positive sample provides weak evidence of  
102 species presence relative to consistent positive samples across replicates over time (Jerde et al.  
103 2011).

104  
105 *eDNA quantitative PCR*

106 We used a species-specific assay for Coastal Cutthroat Trout that targets the cytochrome  
107 oxidase I of the mitochondrial genome for trout in the study area. Each sample was run in  
108 triplicate PCR reactions. PCR was performed using quantitative PCR (qPCR; Biorad). Each 20ul  
109 qPCR reaction contained 6ul of DNA template, 10ul Environmental Master Mix 2.0  
110 (ThermoFisher Scientific, Waltham, MA), 0.2 uM of both forward and reverse primers, 0.2um of  
111 the TaqMan MGB probe, and sterile water. Additionally, each plate contained a four-point  
112 standard curve using DNA obtained from Coastal Cutthroat Trout tissue. Extracted tissue was  
113 quantified using a Qubit Fluorometer (ThermoFisher Scientific, Waltham, MA) and diluted from  
114  $10^{-1}$  ng/ul to  $10^{-4}$  ng/ul. PCR cycling conditions involved an initial denaturation step of 10 min at  
115 95C to activate the HotStart Taq DNA polymerase, followed by 50 cycles of 95C for 15 s and  
116 60C for 60 s. All reaction plates contained a negative control of water and extraction blanks.

117

118 *Electrofishing sampling and physical habitat surveys*

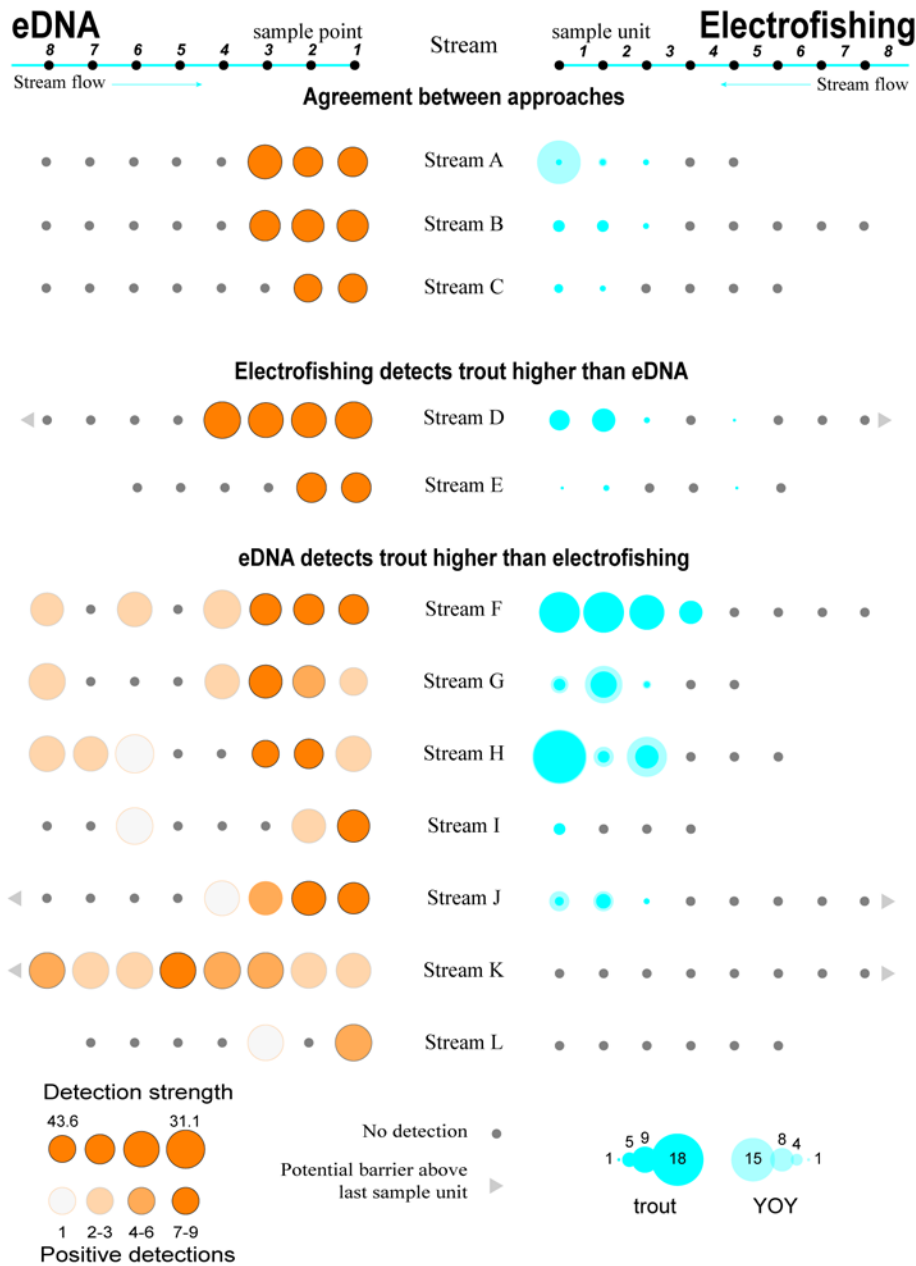
119 After eDNA water samples were collected at the downstream sampling point in each  
120 stream, we sampled the entire 50 m electrofishing unit using standard backpack electrofishing to  
121 determine the end-of-fish. We used a spatially continuous, single-pass backpack electrofishing  
122 approach similar to that described by Torgerson et al. (2004) and validated by Bateman et al.  
123 (2005). but sampling all accessible habitats We electrofished to compare relative abundance data  
124 between both approaches, and consequently, our protocol differs from typical electrofishing to  
125 identify the upper extent of fish where fish are not typically netted (WA DNR 2002).  
126 Electrofishing settings were set to the appropriate settings for each stream. We measured total  
127 length (mm) and weight (g; to tenths) of each fish captured. We processed fish, at least, at each  
128 50-m reach break for a total of 350m of electrofishing per stream.

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

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

Ownership	Location	Stream	Latitude last-fish <sup>1</sup>	Longitude last-fish <sup>1</sup>	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 <sup>1</sup>determined by electrofishing



135  
 136 **Figure 3.** Patterns of agreement and disagreement in Coastal Cutthroat Trout detection between  
 137 methods using eDNA relative abundances and electrofishing over 8 sites across twelve sample  
 138 streams on Weyerhaeuser Company, Port Blakely, and Hancock Forest Management land in  
 139 Washington. In the upper section, we illustrate streams where both methods, eDNA (orange) and  
 140 electrofishing (blue), showed full agreement as to the upper extent of fish. Gray circles represent  
 141 no detection and gray x's represent not sampled for both methods. In the middle section, we  
 142 illustrate streams where electrofishing detected trout upstream of eDNA. Size of the symbols  
 143 represents eDNA detection strength (threshold cycle value: Cq) and fish abundance from  
 144 electrofishing (#/per 50 m sample unit). The lower section shows streams where eDNA detected  
 145 trout above electrofishing. Each row represents a single stream with arrows indicating stream

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

150 *Data Analysis*

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

156  
157 *Occupancy Modeling Approach using broader suite of sites in Washington and Oregon*

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

163 We used a three-level occupancy model EDNAOCCUPANCY in R that uses Bayesian  
164 methods of analysis of Markov Chain Monte Carlo (MCMC) methods of maximum-likelihood to  
165 estimate model parameters [i.e.,  $\Psi(\cdot)\theta(\cdot)p(\cdot)$ ] and include covariates (Dorazio and Erickson  
166 2017). Accordingly, we can estimate fish detection probabilities while also estimating the  
167 conditional probability of detecting trout DNA that may be present in a field sample or qPCR  
168 replicate. The three levels of sampling included aspects of the nested sampling design innate in  
169 eDNA sampling of location (stream x site), field sample, and qPCR replicate. In the model,  $\Psi$   
170 is the probability that the eDNA is present at a location,  $\theta$  is the conditional probability that eDNA  
171 occurred in a replicate sample given that it occurred at the location level, and  $p$  is the conditional  
172 probability of detecting eDNA in a replicate qPCR reaction given that it occurred at the field  
173 sample level.

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

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

199 **Table 2.** Parameter estimates (posterior mean  $\pm$  SE) and model-selection criteria (PPLC and WAIC) for each candidate model of Coastal Cutthroat  
 200 Trout eDNA detections for 31 streams using same protocol. Streams included 12 CMER-funded streams and 19 non-CMER funded streams.  
 201 Values represent either probabilities or estimates of the coefficients of the relationship between the covariate(s) and detection probability of the  
 202 form  $\text{logit}(\Psi) = \alpha_0 + \alpha_1 * \text{covariate} + \alpha_2 * \text{covariate}_2$ , or  $\text{logit}(\theta) = \beta_0 + \beta_1 * \text{covariate} + \beta_2 * \text{covariate}_2$ , or  $\text{logit}(P) = \delta_0 + \delta_1 * \text{covariate}$ .

	Occupancy in location ( $\Psi$ )	Occupancy in sample ( $\theta$ )	Detection in replicate ( $P$ )	PPLC	WAIC
$\Psi(\cdot), \theta(\cdot), P(\cdot)$	0.53 (0.46, 0.59)	0.78 (0.73, 0.83)	0.89 (0.86, 0.91)	190.176	0.3673
<b><math>\Psi(\text{depth+width}), \theta(\text{trout}), P(\text{all fishes})</math></b>	$\alpha_0 = 0.469 (\pm 0.013)$ $\alpha_1 = -0.153 (\pm 0.006)$ $\alpha_2 = 0.593 (\pm 0.012)$	$\beta_0 = 0.930 (\pm 0.004)$ $\beta_1 = 2.331 (\pm 0.025)$	$\delta_0 = 1.102 (\pm 0.001)$ $\delta_1 = 0.191 (\pm 0.001)$	222.008	0.4153
$\Psi(\text{depth}), \theta(\text{trout+all fishes}), P(\text{all fishes})$	$\alpha_0 = 0.649 (\pm 0.016)$ $\alpha_1 = 0.092 (\pm 0.004)$	$\beta_0 = 1.031 (\pm 0.006)$ $\beta_1 = 1.986 (\pm 0.028)$ $\beta_2 = 1.863 (\pm 0.017)$	$\delta_0 = 1.101 (\pm 0.001)$ $\delta_1 = 0.191 (\pm 0.001)$	222.756	0.4158
$\Psi(\text{width}), \theta(\text{trout+all fishes}), P(\text{all fishes})$	$\alpha_0 = 0.635 (\pm 0.011)$ $\alpha_1 = 0.504 (\pm 0.004)$	$\beta_0 = 1.046 (\pm 0.006)$ $\beta_1 = 1.892 (\pm 0.021)$ $\beta_2 = 1.918 (\pm 0.018)$	$\delta_0 = 1.102 (\pm 0.001)$ $\delta_1 = 0.191 (\pm 0.001)$	222.320	0.4158
$\Psi(\text{width}), \theta(\text{trout}), P(\text{all fishes})$	$\alpha_0 = 0.359 (\pm 0.007)$ $\alpha_1 = 0.410 (\pm 0.004)$	$\beta_0 = 0.928 (\pm 0.004)$ $\beta_1 = 2.169 (\pm 0.022)$	$\delta_0 = 1.104 (\pm 0.001)$ $\delta_1 = 0.192 (\pm 0.001)$	221.471	0.4167
$\Psi(\cdot), \theta(\text{trout}), P(\text{all fishes})$	0.63 (0.51, 0.79)	$\beta_0 = 0.938 (\pm 0.004)$ $\beta_1 = 2.231 (\pm 0.029)$	$\delta_0 = 1.104 (\pm 0.001)$ $\delta_1 = 0.194 (\pm 0.001)$	221.540	0.4172
$\Psi(\text{width+depth}), \theta(\text{trout+all fishes}), P(\text{all fishes})$	$\alpha_0 = 0.801 (\pm 0.011)$ $\alpha_1 = 0.851 (\pm 0.010)$ $\alpha_2 = -0.267 (\pm 0.006)$	$\beta_0 = 1.034 (\pm 0.006)$ $\beta_1 = 1.936 (\pm 0.017)$ $\beta_2 = 1.971 (\pm 0.019)$	$\delta_0 = 1.102 (\pm 0.001)$ $\delta_1 = 0.189 (\pm 0.001)$	222.940	0.4174
$\Psi(\text{depth}), \theta(\text{trout}), P(\text{all fishes})$	$\alpha_0 = 0.329 (\pm 0.008)$ $\alpha_1 = 0.122 (\pm 0.002)$	$\beta_0 = 0.936 (\pm 0.004)$ $\beta_1 = 2.184 (\pm 0.026)$	$\delta_0 = 1.104 (\pm 0.001)$ $\delta_1 = 0.193 (\pm 0.001)$	221.562	0.4181

203

204

## 205 Results

206 Three streams (25%) agreed to the upper extent of fish for both approaches (Table 1; Fig. 3).  
 207 Trout eDNA was detected above the last observed fish with electrofishing in seven streams  
 208 (58%) by 50–250 m. Two of these seven streams did not have any trout observed with  
 209 electrofishing (Streams K and L). The most upstream trout detections with eDNA had fewer  
 210 replicate eDNA detections than downstream sites in the same stream, often 1 to 3 of 9 total  
 211 replicates. Two other streams (17%) resulted in fish observed 50–150 m higher with  
 212 electrofishing than eDNA. All study streams had trout detections with eDNA.

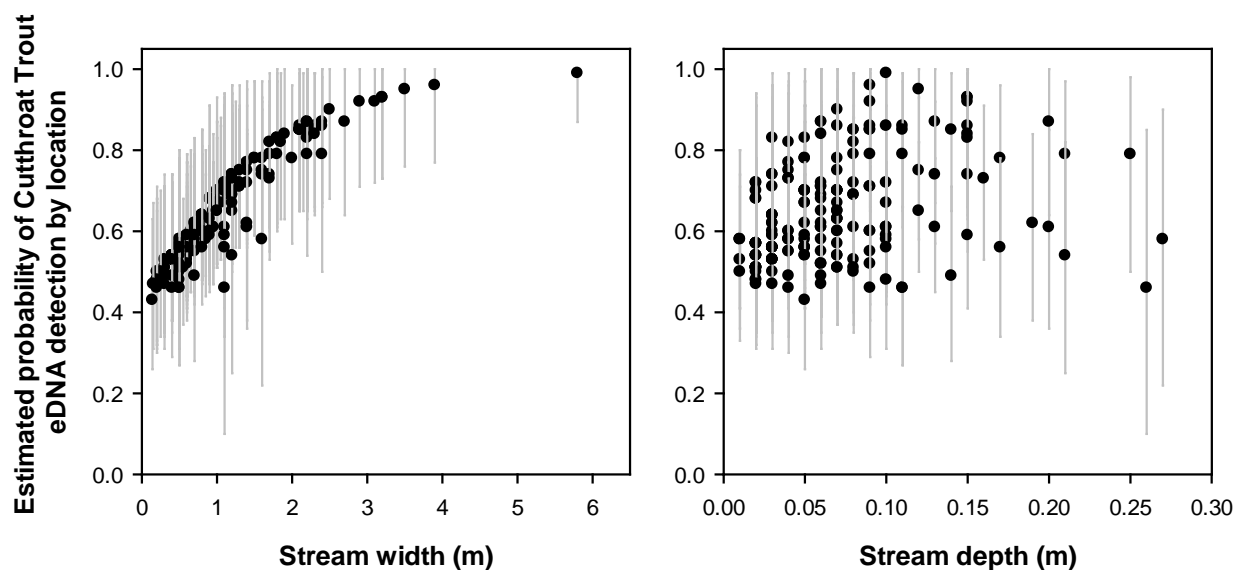
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### 214 *Model results using broader suite of sites in Washington and Oregon*

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

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

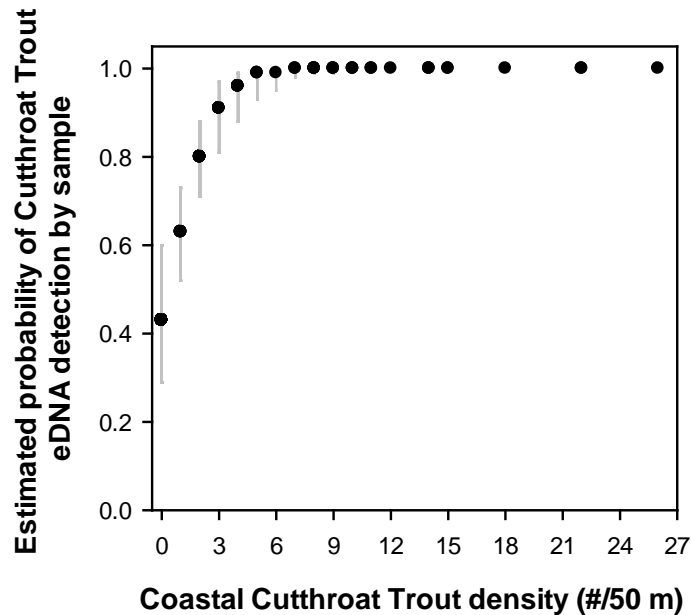
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232  
 233 Figure 4. Estimated probabilities of occurrence of trout eDNA by location ( $\Psi$ ) increases with  
 234 stream width. Location is stream x site. Symbols are estimates of posterior means with 95%  
 235 credible intervals for the model [ $\Psi(\text{depth}+\text{width})$ ,  $\theta(\text{trout})$ ,  $P(\text{all fishes})$ ] described in Table 2.

236





238 **Coastal Cutthroat Trout density (#/50 m)**  
 239 Figure 5. Estimated probabilities of occurrence of trout eDNA in field samples ( $\theta$ ) increases with  
 240 increased trout density from electrofishing. Symbols are estimates of posterior means with 95%  
 241 credible intervals of the model [ $\Psi(\text{depth}+\text{width})$ ,  $\theta(\text{trout})$ ,  $P(\text{all fishes})$ ] described in Table 2.  
 242 Field samples are 1L biological replicates that were taken in triplicate.

243  
 244

## 245 Discussion

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

247

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

260 *Can eDNA be used in addition to electrofishing to determine the end-of-fish?*

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

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

284  
 285 Table 3. Comparison of eDNA versus electrofishing approaches to delimit upper extent of fish.  
 286 **Bold face** denotes positive characteristics of method.

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

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

290 *What are the operational limitations to the use of eDNA to determine the end-of-fish?*  
 291 eDNA warrants inclusion amongst the sampling approaches considered to identify the upper  
 292 extent of fish. We need to continue to push the boundaries of eDNA detections to identify where

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

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

320

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329

### 330 **Literature Cited**

331 Baldigo, B.P., Sporn, L.A., George, S.D. and Ball, J.A., 2017. Efficacy of environmental DNA to  
332 detect and quantify brook trout populations in headwater streams of the Adirondack  
333 Mountains, New York. *Transactions of the American Fisheries Society*, 146(1), pp.99-  
334 111.

335 Bateman, D.S., Gresswell, R.E. and Torgersen, C.E., 2005. Evaluating single-pass catch as a tool  
336 for identifying spatial pattern in fish distribution. *Journal of Freshwater Ecology* **20(2)**:  
337 335-345.

338 Blinn, C. R, and M. A Kilgore. 2001. Riparian management practices: a summary of state  
339 guidelines. *Journal of Forestry* 99(8):11–17.

340 Budy, P., K.B. Rogers, Y. Kanno, B.E. Penaluna, N.P. Hitt, G. P. Thiede, J. Dunham, C.  
341 Mellison, W.L. Somer, and J. DeRito. 2019. Distribution and status of trout and char in  
342 North America. Chapter 7 In: Kershner, J.L., J.E. Williams, R.E. Gresswell, and J.  
343 Lobon-Cervia (eds.), *Trout and Char of the World*. American Fisheries Society,  
344 Bethesda, MD. 777 p.

345 Darling, J. A., and A. R. Mahon. 2011. From molecules to management: adopting DNA-based  
346 methods for monitoring biological invasions in aquatic environments. *Environmental*  
347 *research* 111:978-988.

348 Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E. and Miaud, C., 2012. Improved  
349 detection of an alien invasive species through environmental DNA barcoding: the  
350 example of the American bullfrog *Lithobates catesbeianus*. *Journal of applied ecology*  
351 **49**: 953-959.

352 Dorazio, R.M. and Erickson, R.A., 2018. ednaoccupancy: An r package for multiscale occupancy  
353 modelling of environmental DNA data. *Molecular ecology resources* **18(2)**: 368-380.

354 Evans, N.T., Shirey, P.D., Wieringa, J.G., Mahon, A.R. and Lamberti, G.A., 2017. Comparative  
355 cost and effort of fish distribution detection via environmental DNA analysis and  
356 electrofishing. *Fisheries*, 42(2), pp.90-99.

357 Goldberg, C.S., Strickler, K.M. and Pilliod, D.S., 2015. Moving environmental DNA methods  
358 from concept to practice for monitoring aquatic macroorganisms. *Biological*  
359 *Conservation* **183**: 1-3.

360 Goldberg, C. S., C. R. Turner, K. Deiner, K. E. Klymus, P. F. Thomsen, M. A. Murphy, S. F.  
361 Spear, A. McKee, S. J. Oyler-McCance, R. S. Cornman, and M. B. Laramie. 2016.  
362 Critical considerations for the application of environmental DNA methods to detect  
363 aquatic species. *Methods in Ecology and Evolution* 7:1299-1307.

364 Guillera-Arroita, G., J. J. Lahoz-Monfort, A. R. van Rooyen, A. R. Weeks, and R. Tingley. 2017.  
365 Dealing with false-positive and false-negative errors about species occurrence at multiple  
366 levels. *Methods in Ecology and Evolution* 8:1081–1091.

367 Hunter, M.E., Oyler-McCance, S.J., Dorazio, R.M., Fike, J.A., Smith, B.J., Hunter, C.T., Reed,  
368 R.N. and Hart, K.M., 2015. Environmental DNA (eDNA) sampling improves occurrence  
369 and detection estimates of invasive Burmese pythons. *PloS one*, 10(4), p.e0121655.

370 Itakura, H., Wakiya, R., Yamamoto, S., Kaifu, K., Sato, T. and Minamoto, T., 2019.  
371 Environmental DNA analysis reveals the spatial distribution, abundance, and biomass of  
372 Japanese eels at the river-basin scale. *Aquatic Conservation: Marine and Freshwater*  
373 *Ecosystems*, 29(3), pp.361-373.

374 Jerde, C. L., A. R. Mahon, W. L. Chadderton, and D. M. Lodge, 2011 “Sight-unseen” detection  
375 of rare aquatic species using environmental DNA. *Conservation Letters* 4:150–157.

376 Lee, P, C Smyth, and S Boutin. 2004. Quantitative review of riparian buffer width guidelines  
377 from Canada and the United States. *Journal of Environmental Management* 70:165–180.

378 Levi, T., Allen, J.M., Bell, D., Joyce, J., Russell, J.R., Tallmon, D.A., Vulstek, S.C., Yang, C.  
379 and Yu, D.W., 2019. Environmental DNA for the enumeration and management of  
380 Pacific salmon. *Molecular ecology resources* **19(3)**: 597-608.

381 Penaluna B.E., J.M. Allen, I. Arismendi, T. Levi, T. Garcia, and J. Walter. *In press*. Better  
382 Boundaries: Identifying the upper extent of fish distributions in forested streams with  
383 using eDNA and electrofishing. *Ecosphere*.

384 Peterson, J. T., R. F. Thurow, and J. W. Guzevich. 2004. An evaluation of multipass  
385 electrofishing for estimating the abundance of stream-dwelling salmonids. *Transactions*  
386 *of the American Fisheries Society*, 133:462-475.

387 Rosenberger, A. E., and J. B. Dunham. 2005. Validation of abundance estimates from mark-  
388 recapture and removal techniques for rainbow trout captured by electrofishing in small  
389 streams. *North American Journal of Fisheries Management* 25(4):1395-1410.

390 Roussel, J. M., J. M. Paillisson, A. Treguier, and E. Peti. 2015. The downside of eDNA as a  
391 survey tool in water bodies. *Journal of Applied Ecology* 52:823–826.

392 WA DNR [Washington Department of Natural Resources]. 2002. Section 13: Guidelines for determining  
393 fish use for the purpose of typing waters. Pages M13-1–M13-5 in Washington Forest Practices  
394 Board [n.d.] Forest Practices Board Manual. Olympia, WA: Washington Department of Natural  
395 Resources. Unpublished online manual.  
396 [https://www.dnr.wa.gov/publications/fp\\_board\\_manual.pdf?sop03gu](https://www.dnr.wa.gov/publications/fp_board_manual.pdf?sop03gu)

397 Ostberg, C.O., Chase, D.M., Hoy, M.S., Duda, J.J., Hayes, M.C., Jolley, J.C., Silver, G.S. and  
398 Cook-Tabor, C., 2019. Evaluation of environmental DNA surveys for identifying  
399 occupancy and spatial distribution of Pacific Lamprey (*Entosphenus tridentatus*) and  
400 *Lampetra* spp. in a Washington coast watershed. *Environmental DNA*.  
401 <https://doi.org/10.1002/edn3.15>

402 Pilliod, D.S., Goldberg, C.S., Arkle, R.S. and Waits, L.P., 2013. Estimating occupancy and  
403 abundance of stream amphibians using environmental DNA from filtered water samples.  
404 *Canadian Journal of Fisheries and Aquatic Sciences* 70(8): 1123-1130.

405 Price, A.L. and Peterson, J.T., 2010. Estimation and modeling of electrofishing capture  
406 efficiency for fishes in wadeable warmwater streams. *North American Journal of*  
407 *Fisheries Management* 30(2): 481-498.

408 Sigsgaard, E.E., Carl, H., Møller, P.R. and Thomsen, P.F., 2015. Monitoring the near-extinct  
409 European weather loach in Denmark based on environmental DNA from water samples.  
410 *Biological Conservation* 183: 46-52.

411 Torgersen, C.E., Gresswell, R.E. and Bateman, D.S., 2004. Pattern detection in stream networks:  
412 quantifying spatial variability in fish distribution. *GIS/spatial analyses in fishery and*  
413 *aquatic sciences* 2: 405-420.

414 Wilcox, T.M., McKelvey, K.S., Young, M.K., Sepulveda, A.J., Shepard, B.B., Jane, S.F.,  
415 Whiteley, A.R., Lowe, W.H. and Schwartz, M.K., 2016. Understanding environmental  
416 DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus*  
417 *fontinalis*. *Biological Conservation* 194: 209-216.

418 Wilson, C., E. Wright, J. Bronnenhuber, F. MacDonald, M. Belore, and B. Locke. 2014.  
419 Tracking ghosts: combined electrofishing and environmental DNA surveillance efforts  
420 for Asian carps in Ontario waters of Lake Erie. *Management of Biological Invasions*  
421 5:225–231.  
422