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July 20, 2021

TO: Forest Practices Board

FROM: Joe Shramek, Forest Practices Division Manager JPS

SUBJECT: **Transmittal: Identifying Distribution Boundaries at the Upper Extent of Fish in Streams Using Environmental DNA Study**

Because the adaptive management program administrator position is vacant, it is my pleasure to formally transmit the study entitled: *Identifying Distribution Boundaries at the Upper Extent of Fish in Streams Using Environmental DNA*. At its June, 2021 meeting, TFW Policy formally considered and acted on the CMER-approved final study report and CMER's answers to the first six questions of the Framework for Successful Policy/CMER Interaction (Appendix B of Board Manual, M22-28).

This was an exploratory study that was opportunistically pursued under a cost-share agreement with the USDA Forest Service Pacific Northwest Research Station (USFS) to add sites from Washington to an environmental DNA (eDNA) study that was being conducted in Oregon. The Washington sites were chosen to test the eDNA sampling methodology where electrofishing survey work had been previously scheduled by industrial forest landowners.

The reason for joining the USFS and industrial landowners in this study was primarily to evaluate the methodology used to evaluate in general how eDNA sampling can contribute to the demarcation of fish- and non-fish-habitat, and to inform CMER about how to best incorporate eDNA sampling into future CMER studies (for example, PHB validation, Default Physical Habitat).

As an opportunistic exploratory study, neither the methods nor the final report were submitted to our independent peer review process. The final report was conducted by the principal investigator and reviewed and approved by CMER.

This study raised a number of concerns regarding the specific methods employed. Although identifying problems and process gaps is never ideal, in this situation they are consistent with the intention to use this effort and its challenges as lessons learned that can be applied in future CMER-developed studies. That said, it was a heavy lift to for CMER to develop consensus responses to its six questions for Policy. This experience should be used in the future to temper the enthusiasm to which CMER contributes to work outside of its own processes and control. Taking the time to understand more specifically the what, when, where, and how before committing AMP time and funding to a project or study is time well spent.

Policy concluded that it was not intended, nor is it appropriate, to apply the results of this study to propose that the Board take any formal action with regard to established forest practices rules or board manual guidance. CMER is expected to include eDNA methods in its future water-typing-

related study efforts, and when that happens it will both apply the lessons learned from this endeavor and submit those study designs to strenuous peer review.

After reviewing the eDNA Findings Report and Six Questions documents, *Policy agreed by consensus to recommend that the Board take no formal action in response to this study.* Policy suggests that the Board encourage CMER to continue to develop and evaluate this tool through future CMER studies as a potential tool for use within the forest practices program.

JS
Attachment

DRAFT answers to CMER's Six Questions for the pilot report -

Identifying Distribution Boundaries at the Upper Extent of Fish in Streams Using Environmental DNA

May 19, 2021

1. **Does the study inform a rule, numeric target, performance target, or resource objective?**

   No, not directly. This effort was designed to contribute information to a larger study yet to be scoped by CMER (see CMER work plan, page 19). The originally defined purpose of this project was focused primarily on methods. Per the 'Fish Habitat Detection Using eDNA Project Description Worksheet' from 2017, the project was implemented to: (1) evaluate how eDNA sampling can contribute to the demarcation of fish- and non-fish-habitat waters, (2) investigate how this tool can contribute to the better characterization of instream habitat, the evaluation of relative abundance, and distribution of several aquatic species, and (3) provide a cost and efficiency comparison of eDNA analysis methods: *e.g.* qPCR probe or multi-species screen (MP-eDNA).

2. **Does the study inform the Forest Practices Rules, the Forest Practices Board Manual guidelines, or Schedules L-1 or L-2?**

   No, not directly.

3. **Was the study carried out pursuant to CMER scientific protocols (i.e., study design, peer review)?**

   No. This study was approved by Policy and the FP Board as a cooperative cost-sharing venture with the Pacific Northwest Research Station (PNWRS). Therefore, the standard CMER scoping, alternatives analysis, study design review, ISPR process, and other elements outlined in the CMER Protocols and Standards Manual were not carried out. The implementation steps necessary to carry out the study were performed by Dr. Brooke Penaluna (PI) and the project team with the Pacific Northwest Research Station, United States Forest Service. The study design was revised to provide data and analysis to support the critical questions outlined in the CMER work plan (2021-23) under the Washington Department of Natural Resources Adaptive Management Program. CMER participation was opportunistic and done in collaboration with WA industrial landowners who donated the sites and corresponding electrofishing data. Washington study sites were chosen specifically to test the eDNA sampling methodology where previously scheduled electrofishing survey work was already being conducted by industrial landowners (ISAG budget memo to CMER, 2018). Final analysis was conducted by the Principal Investigator and reviewed by ISAG and CMER members.

4. **4A - What does the study tell us?**

   - This study (experience) tells us that even exploratory studies within CMER need to be administered with more oversight and accountability for deliverables to fulfill the needs of the AMP.

- This study tells us that variability exists in where/when positive trout eDNA detections align with confirmed trout presence through e-fishing, but the reasons for that variability are not clear. In Results (lines 206-212) the author reports that; the uppermost positive trout eDNA detection agreed with the uppermost detected trout identified by e-fishing in 25% of sites, the uppermost trout identified by e-fishing was upstream from the uppermost positive trout eDNA detection in 17% of sites, and the uppermost positive trout eDNA detection was upstream from the uppermost detected trout identified by e-fishing in 58% of sites. To address this reported variability, the author does acknowledge in the Discussion (lines 251-259) that, "*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).*
- This study tells us that, "*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.*" (Results – Lines 219-222). In addition, the author addresses the issue of fish density relative to a positive eDNA test result in the Discussion (Lines 267-268), by stating, "*In most cases, it is able to detect trout in low densities, but sometimes it also misses them.*"
- Table 3 (below) in the Discussion (Lines 285-286) provides a direct comparison of eDNA versus electrofishing approaches to delimit upper extent of fish. **Bold** text in the comparison table denotes positive characteristics of a given method where a difference exists. Three of the metrics ('offers data instantaneously', 'identifies exact time and place of fish', and 'potential for false positives') compared in the table that are critical to the logistical practicality and ability to implement the methods for water typing purposes identify a benefit of e-fishing over the use of eDNA.

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

4B - What does the study not tell us?

- This study does not tell us about the logistical practicality or ability to implement eDNA as a stand-alone water typing tool in streams. In Methods (lines 50-53) the author states, "*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…*". Additionally, in the Discussion (lines 295-296) the author states, "*The effectiveness of eDNA depends on investigators being informed of the potential location of last-fish to know where to start sampling…* ". In summary, without the previously existing information on fish distribution the author would not have known where to implement eDNA sampling within the subject watersheds.
- This study does not tell us about the relative detectability of specific eDNA and e-fishing protocols used in this study, and detectability is not considered in the comparisons of the two methods. In Methods (lines 99-103), the author states, "*We consider detection of trout DNA in a sample as a positive signal from a single replicate out of 9 possible replicates*" and that, "*a single positive sample provides weak evidence of species presence relative to consistent positive samples across replicates over time…*". However, in the Discussion (lines 308-313), the author suggests that a higher threshold (more replicates) could define a positive eDNA detection as part of a decision-making framework. Although the author recognizes that the single replicate method used in the study is a low threshold of detection, a potential bias for eDNA detection is not acknowledged when comparing results to e-fishing. For example, the e-fishing protocol required netting fish (catch) for detection, which is a significantly higher threshold of detection than the simple visual detection required for WDNR protocol. Consequently, the detection comparison between methods is biased given the low threshold used for eDNA detection and high threshold for e-fishing detection.
- This study does not provide information about the number of streams (and, importantly, harvest units) required for implementing eDNA as a tool for assessing fish presence. E-fishing assumes that detection probability, or the probability of detecting a fish given the occurrence of fish in a stream, is very close to 1, an assumption that has been validated by previous work. Use of eDNA as a fish detection tool requires estimation of the probability of detection in a sub-sample of stream water given that a species' eDNA was present in the sample. To estimate eDNA detection probability requires that a number of streams be sampled to have a sufficient amount of data to fit the statistical model that estimates the detection probability. Unfortunately, the number of streams required to fit the statistical model was not evaluated in this project. For e-fishing, surveys of other streams are not required to make a determination about fish presence (given the general assumption about detection probability of fish using e-fishing protocols). More work is required to determine how many streams are sufficient to estimate eDNA detection probabilities at desired levels of confidence.
- This study does not tell us about how stream conditions and/or habitat factors (e.g., discharge, depth, temperature) may influence e-fishing detection, nor does the author include this information for individual study sites in the report. Also, the author does not discuss how site-specific conditions could potentially influence e-fishing detection and/or the location of the last detected fish that may create potential bias in comparisons of

findings. For example, the study data file from Penaluna (2020) shows that on one day of e-fishing the water temperature at site F was <6° C. Research shows low detection/catch at such low temperatures (Zale et al. 2012).

- Without repeat surveys, both methods fail to account for seasonal and annual variability in last fish location. *(When comparing e-fishing last fish locations with eDNA results, one must consider potential biases for both methods (e.g., seasonal and annual variability in last fish location, and variability in local instream habitat conditions from survey to survey)).*
- This study does not take into account the presence of the eDNA crew in the stream channel ahead of the e-fishing crew, possibly causing fish to move (thereby influencing fish presence/absence in a given stream segment), and therefore impacting study results. The lack of recognition for this potential impact was not presented in Results, despite the fact that the author briefly acknowledges the potential for this issue in the Discussion (Lines 271-272).
- This study does not tell us how the size of fish at study sites may have influenced e-fishing detection rates, despite acknowledging in the Discussion (lines 275-276) that, "*At its optimal, standard backpack electrofishing is most efficient for larger fish …*", nor does the author include fish size information for individual study sites in the report. Research shows that most fish in headwaters are small (typically <150 mm), thus e-fish detection at these sites may be greatly reduced compared to larger fish (Zale et al. 2012).
- This study does not tell us about the persistence of trout eDNA in the environment, nor does it provide any information about how far trout eDNA may travel in a stream system. In the Discussion (Lines 297-301) the author specifically states, "*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*.".

5. What is the relationship between this study and any others that may be planned, underway, or recently completed?

Genetic material shed by all living organisms and found in the environment is referred to as environmental DNA (eDNA). In the last two decades, noninvasive genetic sampling has been recognized as a potentially effective conservation and management tool for monitoring the presence and distribution of specific species and to assist in quantifying biodiversity within a specific environmental system. Environmental DNA sampling methods are being developed that may contribute to more accurate demarcation between fish- and non-fish-habitat waters.

There is a rapidly growing body of research and methodology reports concerning the application of eDNA analysis that should be consulted as CMER moves forward in the development of projects aimed to test eDNA as a tool in the water typing toolkit. Some key questions that could potentially be answered by literature review and/or collaborative projects include the following (CMER work plan, 2021-2023):

- How does eDNA sampling compare with electrofishing for overall effectiveness, costs, and accuracy for identifying fish presence?

- What sampling conditions are conducive to accurately and consistently identify fish presence?
- Could eDNA sampling be used to better characterize fish presence as it relates to fish habitat?

Previously published research by Cole and Lemke (2006) assessing variability in fish distribution with electrofishing found that fish move upstream and downstream as habitat condition change seasonally and annually. After conducting repeat e-fishing surveys on the same fish-bearing streams in spring 2005 and again in summer 2005 Cole and Lemke found:

> "*Distance between spring 2005 terminal last-fish points and corresponding summer last-fish points ranged from -321 to 290 m and averaged -6.7m (SD ± 73.7 m). The average absolute distance between spring terminal points and corresponding summer points was 89.8 m. Upstream and downstream shifts occurred in nearly equal proportions. Spring 2005 terminal last-fish points did not change from spring to summer at 11 of 55 locations and, when movement occurred (in either direction), the terminal last-fish point had shifted by 25 m or less at an additional 18 of 55 (33%) spring 2005 terminal points. Last fish shifted by more than 100 m in either direction at 9 of 55 (16%) locations and shifted by more than 200 m at only four locations with the largest two movements of -321 m and 290 m. In summer 2005 no fish were sampled from six of 55 channels (11%) that supported fish use in the spring.*"

In May 2020, a 'CMER Water Typing Strategy' memo was delivered to the Forest Practices Board. This memo included recommendations for how to proceed with the 'PHB Validation' (PHB), 'Default Physical Criteria (DPC)' and 'Map-based Lidar Model' (LiDAR Model) studies. One of these recommendations (Recommendation 5) was specifically focused on eDNA, and reads:

> *"There is potential for eDNA (Environmental DNA) to be included as an added element to the PHB and/or DPC studies, however, continued investigation of eDNA as a prospective water typing tool should not necessarily be limited to work within these other studies."*

In February 2021, a 'Update to the WA Forest Practices Board on Water Typing Projects' memo was delivered to the Forest Practices Board. That memo included the following language:

> *"...ISAG has postponed work on this recommendation: Additionally, CMER is currently in the process of finalizing review of the eDNA report that might further inform the extent to which the PHB and DPC studies will lend themselves to the inclusion of an eDNA element."*

6. **What is the scientific basis that underlies the rule, numeric target, performance target, or resource objective that the study informs? How much of an incremental gain in understanding do the study results represent?**

This developmental study was not intended to and does not inform a rule, numeric target performance target, or resource objective. The intent of this work was to assess a process/method, and to help inform if/how eDNA may be; (1) further investigated in additional, broader scale eDNA research through CMER, and/or (2) included as a component of other proposed CMER research (PHB, DPC, etc.).

If not already done so within the answers to the six questions above, provide the technical implications/recommendations resulting from the study. Examples of areas on which to comment include:

- **Issues not resolved with author during review process:**

  - On at least one ownership, the eDNA and electrofishing teams might have been looking at different streams. This issue was mentioned by a landowner representative at the time results were presented; however, while follow-up clarification was requested, none was provided.
  - Clarification was sought from landowners via the author regarding why there were no samples taken at some stations. Whatever came of this discussion, the author chose not to share the information with the reviewers or the readers.
  - Regarding the above item, we asked that the 'X' symbols in Figure 3 be colored differently for "could not sample" vs. "did not sample". Rather than address the issue, the author eliminated the 'Xs', drawing attention away from the fact that no samples were taken in some locations (though the figure caption still refers to these points).
  - A reviewer requested a summary table of the stream physical characteristics data described in lines 129-132 of the final report to provide context for the reported fish survey results. The author agreed to provide this information, but Table 1 does not include <u>any</u> physical stream characteristics, including BFW or gradient, which provide the basis for much of the rule language related to water typing.
  - These issues and others led to ambiguous study results and contributed to different caucus interpretations of the validity of study results.

- **The author provides insufficient detail about model identification, fitting, selection, assessment of fit, and interpretation:**

  - With the sample size used in the analysis (assuming they fit a model with 31 sites, but sample size of analysis is unclear), a model with 6-8 parameters is overfit and the estimates may provide an inaccurate summary of eDNA detection probabilities.
  - Also, the authors did not provide enough information to support the selection of a model to make inference. For example, Table 2 provides selection criteria (PPLC and WAIC)

for each model but does not provide information on the number of estimable parameters for each model (please see *Anderson, D.R. and K.P. Burnham. 2002. Avoiding pitfalls when using information-theoretic approaches. Journal of Wildlife Management 66: 912-918* for required information for model selection criteria).

- The author does not describe how they addressed potential spatial correlation among the eight samples in each stream. The author refers readers to Dorazio and Erickson 2018 (*Dorazio, R.M. and R.A. Erickson. 2018. EDNAOCCUPANCY: an R package for multiscale occupancy modelling of environmental DNA data. Molecular Ecology Resources 18: 368-380*) but this paper does not discuss how spatial auto-correlation may affect parameter estimates in the multiscale model. In a separate application of the same statistical model, Mordecai et al. 2011 (*Mordecai, R.S., B.J. Mattsson, C.J. Tzilkowski, and R.J. Cooper. 2011. Addressing challenges when studying mobile or episodic species: hierarchical Bayes estimation of occupancy and use. Journal of Applied Ecology 48: 56-66*) suggested that random effects could be specified to account for spatial dependence among the sample sites in each stream.

The upshot of leaving these issues unaddressed is that the parameter estimates may not be accurate. Specifically, the estimates of detection may not reflect the true values of the parameters and the precision of the estimates may under-estimate uncertainty.

- **Additional lessons learned:**

  - Genetic markers did not cover all the species likely to be encountered at the study sites which may have led to some of the discrepancies observed between last fish locations determined by eDNA versus electrofishing.
  - Stream profiles and tabular data illustrating where eDNA and electrofishing end of fish points fall relative to each other would provide better context for interpreting the results.
  - More clearly written protocols and field methods, adequate training, oversight, and some measure of accountability for adherence might have reduced uncertainty and made results of the study less ambiguous.
  - Developmental studies such as this are intended to inform future work and help to refine sampling protocols, and by design are often unsuitable for assessing variability, calculating sample sizes, and conducting power analyses for development of subsequent studies.

References

Cole M.B. and J.L. Lemke. 2006. Seasonal variability in the upper limit of fish distribution in eastern Washington stream. Cooperative monitoring, evaluation and research committee, Washington Department of Natural Resources, Olympia WA.

Fisheries Techniques, third edition. 2012. Edited by A. V. Zale, D. L. Parrish, and T. M. Sutton. American Fisheries Society, Bethesda, Maryland.1,009 pages.

Instream Science Advisory Group. (2018, February 20). Fish/Habitat Detection Using eDNA Project (Stream Typing Rule Group Strategy (5.1.4). [Memorandum]. Department of Natural Resources, Adaptive Management Program.

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

By:
Brooke Penaluna

May 2021

CMER #2021.05.25

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Full Reference

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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

This work was developed with public funding, including contracts 93-100869. As such it is within the public use domain. However, the concept of this work originated with the Washington State Forest Practices Adaptive Management Program and the authors. As a public resource document, this work should be given proper attribution and be properly cited.

Approved by CMER 05/25/2021

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 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.

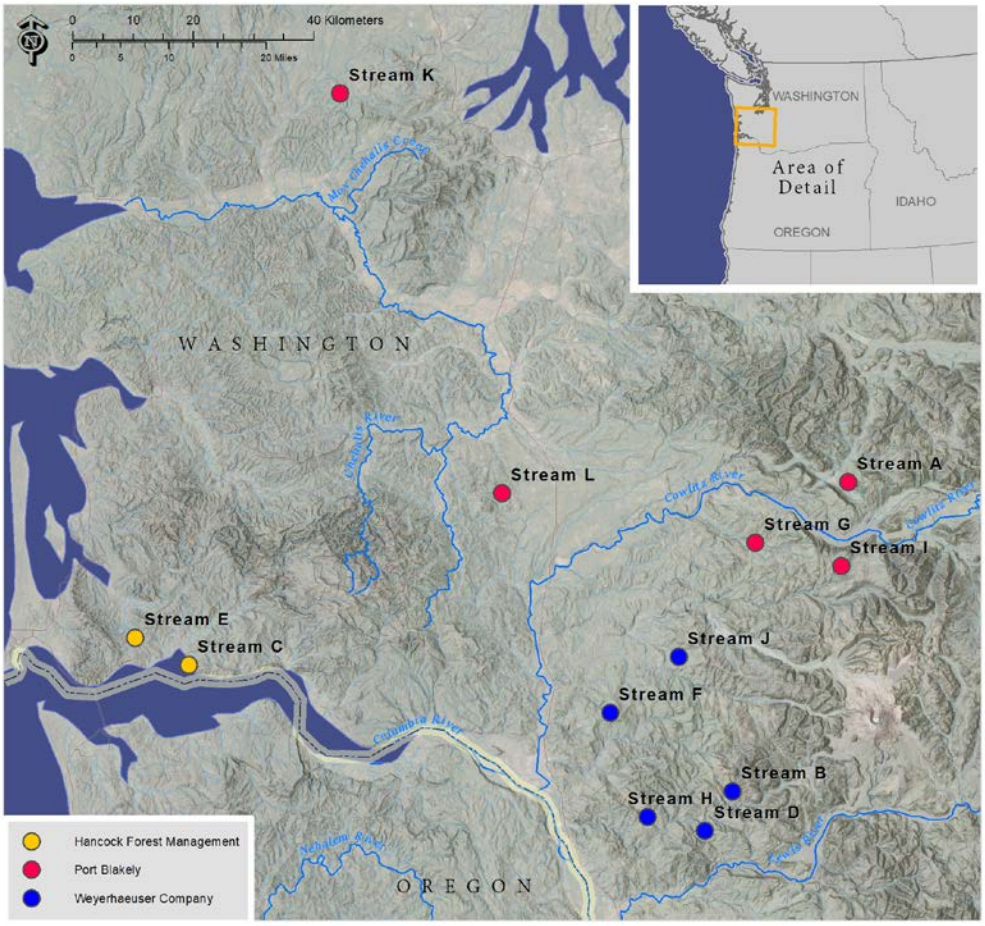

Figure 1. Map of twelve study streams in Southwest Washington. At each stream, the upper extent of fish was evaluated with electrofishing and eDNA.

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.

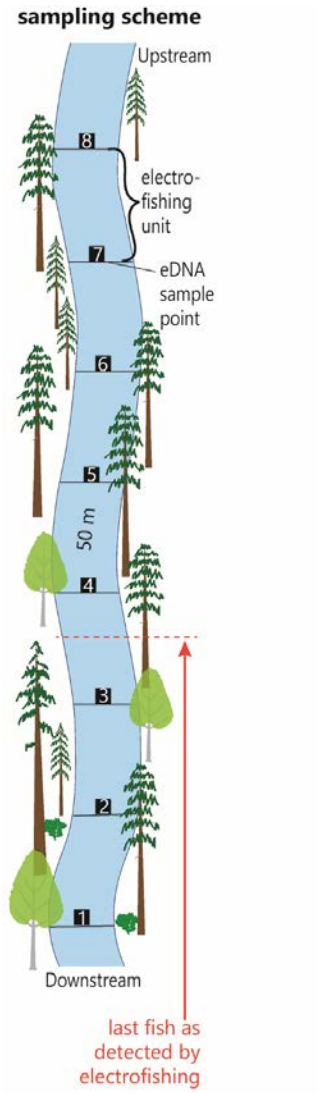

Figure 2. Schematic of eDNA sampling collection, including 8 eDNA sampling points at the downstream end of each electrofishing unit. Generally, the last-fish detected by electrofishing fell between sites 3 and 4, except for streams C, D, E, F, and I.

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

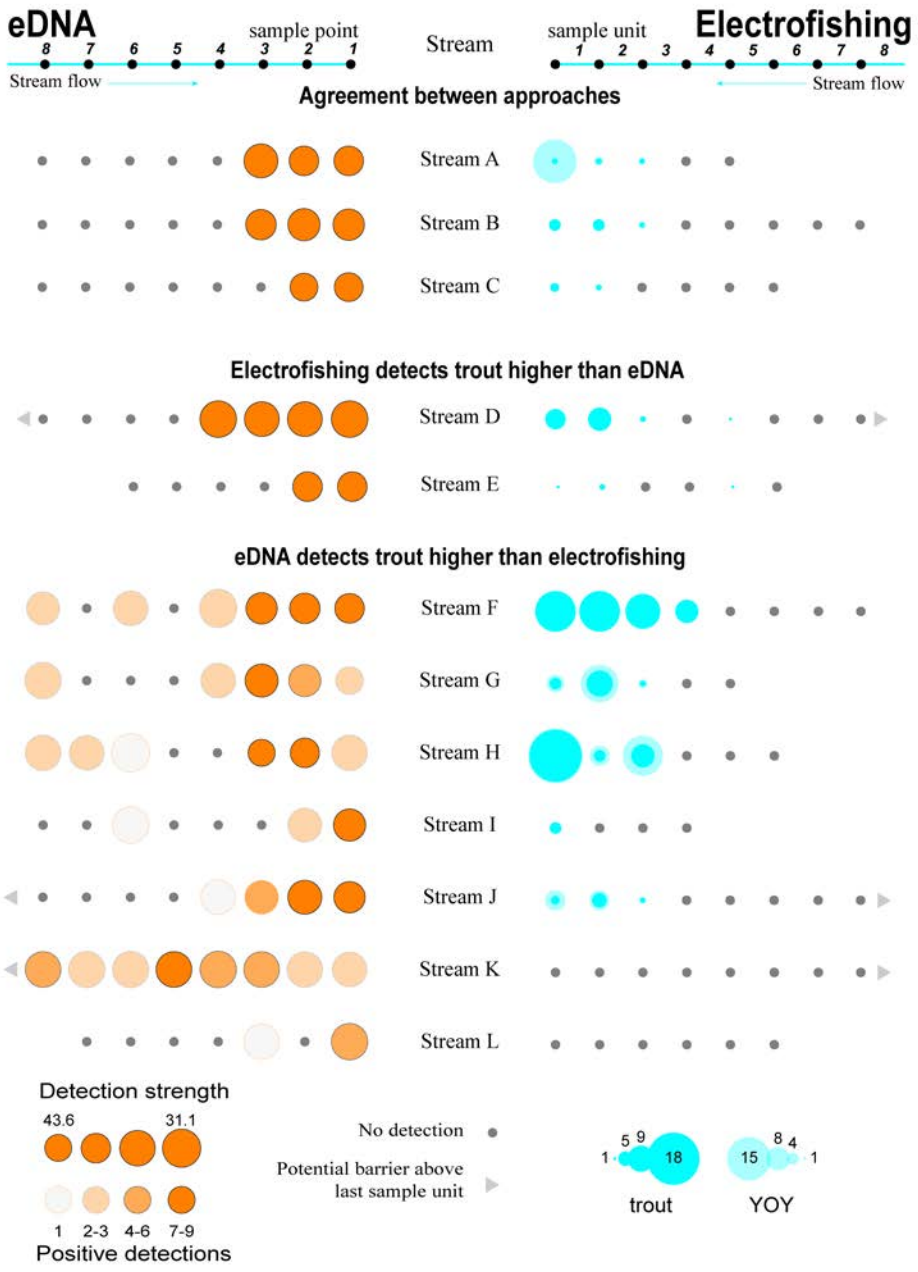

Figure 3. Patterns of agreement and disagreement in Coastal Cutthroat Trout detection between methods using eDNA relative abundances and electrofishing over 8 sites across twelve sample streams on Weyerhaeuser Company, Port Blakely, and Hancock Forest Management land in Washington. In the upper section, 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

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*.

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.

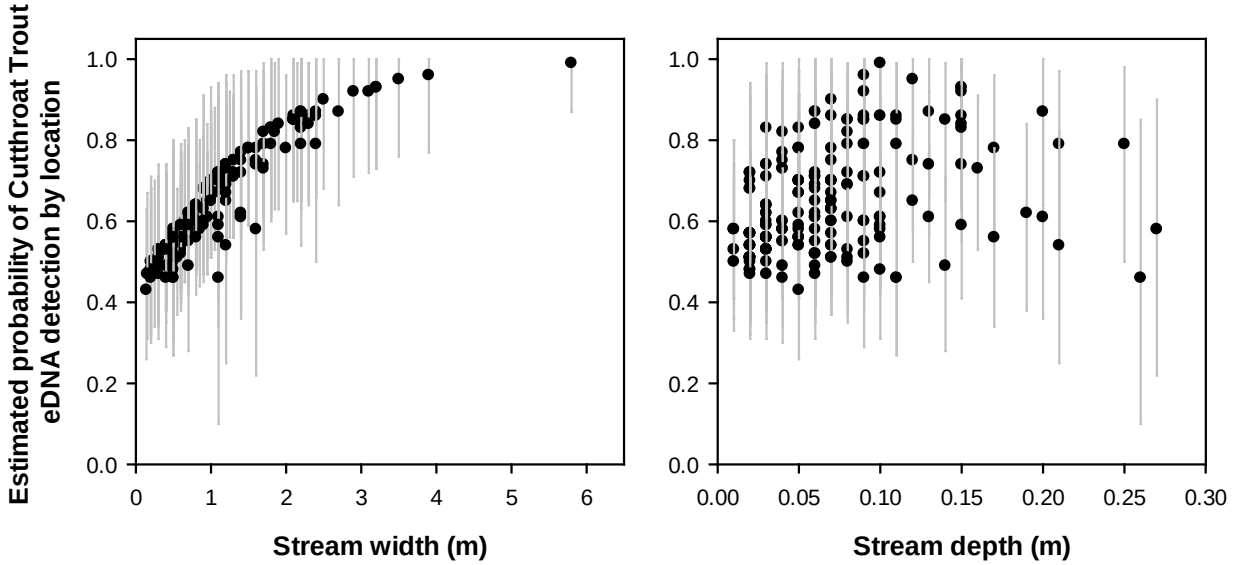

Figure 4. Estimated probabilities of occurrence of trout eDNA by location (Ψ) increases with stream width. Location is stream x site. Symbols are estimates of posterior means with 95% credible intervals for the model [Ψ(depth+width), θ(trout), P(all fishes)] described in Table 2.

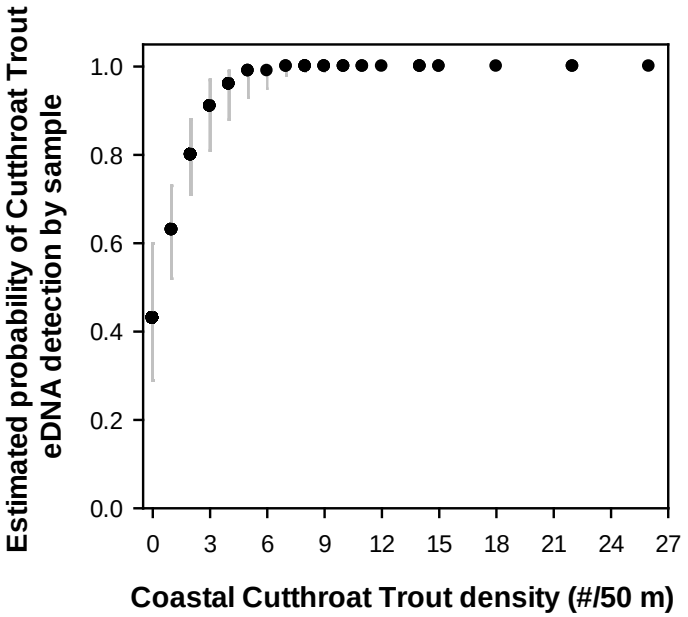

Figure 5. Estimated probabilities of occurrence of trout eDNA in field samples (θ) increases with increased trout density from electrofishing. Symbols are estimates of posterior means with 95% credible intervals of the model [Ψ(depth+width), θ(trout), P(all fishes)] described in Table 2. Field samples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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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. *Canadian Journal of Fisheries and Aquatic Sciences* ***70*****(8)**: 1123-1130.

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

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

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

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

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