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.