Preliminary Assessment of Pacific Lamprey Environmental DNA Samples from Western Washington Rivers.

Final Report
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Introduction/Background

Pacific Lamprey (*Entosphenus tridentatus*) was once a historically widespread anadromous fish species important to freshwater ecosystems and to Native American tribes along the West Coast of North America from California to Alaska (USFWS 2004). Pacific Lamprey have experienced range-wide declines, with persisting isolated populations at risk of extinction (Close et al. 2002; Luzier et al. 2011). The Pacific Lamprey, Western Brook Lamprey (*Lampetra richardsoni*), and Western River Lamprey (*L. ayresii*) were petitioned for protection under the Endangered Species Act in 2003; however, due to limited range-wide distribution and abundance data, the species was deemed ineligible for listing (USFWS 2004). In 2012, several tribes and the U.S. Fish and Wildlife Service (USFWS), along with other state, local, and federal agencies committed to the Pacific Lamprey Conservation Initiative (PLCI; www.pacificlamprey.org). Techniques that can quickly and accurately detect species at low densities and distinguish between species are essential to further inform lamprey conservation efforts. One such effort is using environmental DNA (eDNA) samples for species-specific detection to help determine species presence/absence across the landscape (Carim et al. 2017; Ostberg et al. 2018, 2019).

Recently, occupancy models have emerged as a useful tool for estimating the distribution of fish species that are patchily distributed and may be difficult to sample, including Pacific Lamprey (Reid and Goodman 2015). Environmental DNA represents another useful tool for determining the distribution of species that are difficult to sample using traditional methods (Goldberg et al. 2011). One particularly useful attribute of aquatic eDNA surveys is that this technique allows large areas to be surveyed in a fraction of the time required by traditional methods (Goldberg et al. 2016). Several studies have suggested that eDNA surveys can be more efficient and sensitive than physical sampling gear (e.g., electrofishing, seining), although limitations of this method exist, and data must be interpreted correctly (Goldberg et al. 2016). Environmental DNA detection methods have been developed for Pacific Lamprey and used to fill critical data gaps in their distribution (Carim et al. 2017; Ostberg et al. 2019). Recently, several studies have utilized a combination of traditional field sampling and eDNA surveys to inform occupancy models for species of conservation concern where data on distribution are limited (Schmelzle and Kinziger 2016; Sutter and Kinziger 2019; Smith and Goldberg 2020).

Previously USFWS genetics labs collaborated on an effort to validate native species eDNA markers. Pacific Lamprey was the focus of one such effort in 2021. The USFWS Whitney Genetics Lab aimed to validate two published Pacific Lamprey assays currently in use across the West Coast of North America for eDNA detection in Pacific Northwest watersheds (Hannah and DeHaan 2023). Results indicated that two separate markers developed by Ostberg et al. (2018) and Carim et al. (2017) were highly specific, sensitive, and efficient at amplifying Pacific Lamprey DNA from environmental samples.

The Whitney Genetics Lab (WGL) partnered with the Western Washington Fish and Wildlife Conservation Office (WWFWCO) to help process and analyze eDNA samples collected from watersheds in coastal Washington and the Puget Sound for the population assessment components of the regional management unit's regional implementation plans (Plumb 2019). The objective of this project was to screen preliminary eDNA filter samples collected in Puget Sound and coastal Washington watersheds in 2021 for Pacific Lamprey DNA presence. Analysis

of these samples will help fill data gaps in the distribution of Pacific Lamprey and help inform future eDNA monitoring efforts for this species in Puget Sound and coastal Washington.

Methods

eDNA sample collection

Environmental DNA samples were collected at regular spatial intervals in HUC8s within the Coastal Washington and Puget Sound Regional Management Units identified in the PLCI (see Appendix A and B for sampling locations). At each site, 5 L of water was filtered through glass microfiber filters with a 1.5 µm pore size following protocols developed by the U.S. Forest Service Rocky Mountain Research Station (Carim et al. 2017). If a filter became clogged before a full 5 L of stream water could be processed, additional filters were used for the same site to reach a total volume of 5 L. Samples were collected below a riffle or in a well-mixed portion of the flow, typically the center of the main current near the surface. Field blank samples (distilled water) were randomly taken every 5–10 samples to test for evidence of field contamination. Filters were preserved with silica desiccant beads and stored at -20° C until laboratory analysis. Filters were shipped to WGL in December of 2022.

DNA Extraction

Once samples arrived at the laboratory, they were stored in a -80°C freezer to preserve samples until DNA extraction. DNA was extracted from whole filters using a modified version of the IBI Scientific gMAX extraction kit (IBI Scientific, Dubuque, IA) with Qiagen Lyse and Spin baskets. Whole filters were placed into a lyse and spin basket with 350 µL of GSB buffer and 35 µL of Protinase K and incubated at 60°C for approximately 1 hour. The lysate was spun out of the basket at 18,000 rpm for 1 minute and then we followed the standard IBI gMax extraction protocol for tissue samples with a final elution of 200 µL. For samples with more than one filter collected in the field, filters were processed separately during the incubation/digestion phase of DNA extraction and then the lysate from all filters was combined into a single spin column for further processing. Extracted DNA was stored in a -20°C freezer until qPCR took place. All DNA extraction batches included an extraction positive sample (Pacific Lamprey DNA tissue) to verify that extraction procedures were successful and an extraction negative sample (deionized water only) to ensure that there was no contamination during DNA extraction. All DNA extractions were performed in a room dedicated to DNA extraction (i.e., no amplified DNA was present in this room).

qPCR

All samples were analyzed using two qPCR assays to determine the presence of Pacific Lamprey DNA. These assays target the cytochrome oxidase I gene (PLamp; Carim et al. 2017) and the cytochrome b (EntTri; Ostberg et al. 2018) gene on the Pacific Lamprey mitochondrial genome. qPCR master mixes were set up in a room dedicated to reagent prep. Final master mix reagent concentrations were as reported in Hannah and DeHaan (2023; Appendix C). Template DNA and master mixes were added to PCR plates using a liquid handling robot (Eppendorf EP motion 5075). We ran eight molecular replicates for each sample. Each qPCR plate contained a five-point standard curve with 10, 50, 250, 1250, and 6250 copies/µL of synthetic Gblock DNA (IDT), four PCR negatives (PCRN; 3 µL of deionized water instead of template DNA), and two PCR positives (PCRP; 3 µL of extracted DNA from Pacific Lamprey tissue samples). Cycling

conditions for each assay were as follows: 10 minutes at 95°C followed by 45 cycles of 15 seconds denaturing at 95°C and 30 seconds annealing and extension at 60°C. Samples that crossed the background fluorescence threshold limit between 15 and 45 cycles were considered a positive detection. Any sample with at least one positive qPCR replicate was considered a positive sample. We tested all samples for evidence of PCR inhibition using TaqMan Exogenous Internal Positive Control (IPC) kits (ThermoFisher) following the manufacturer's protocols. All samples were run three times to determine the mean Cq value for the IPC. Any sample with a Cq value that was one cycle greater (ΔCq) than the IPC for a blank sample (IPC and deionized water only) was determined to be inhibited.

Results

A total of 70 water samples were collected across 23 streams between July 29 and September 28, 2021. For most sites, a single filter was required to process 5 L of water. Eight samples (21-003, 21-004, 21-020, 21-022, 21-023, 21-059, 21-060, 21-061) required two filters to process 5 L of water, and four samples (21-068, 21-069, 21-070, 21-071) required three filters to process 5 L of water.

A total of nine field blanks were collected, and none of those had positive detections. None of the lab control samples showed unexpected results either (i.e., all PCR negatives were negative, all PCR positives were positive, etc.). This indicates there was no evidence of field or lab contamination during sample collection and processing. Three of the samples had a positive detection at one of the qPCR assays (Table 1). Sample 21018 was positive for the PLamp assay, and samples 21047 and 21050 were positive for the EntTri assay. Those samples were collected from the Copalis River, Lyre River, and Sekui River, respectively (Appendix A and B). All samples with positive detections had only a single molecular replicate (out of eight) that was positive. All other samples were negative for both assays. Inhibition testing showed that one sample (21017 – a field blank) showed slight evidence of inhibition (ΔCq = 1.077).

Table 1. Samples that showed a positive eDNA detection for assays used in this study. The number of replicates for each assay represents the number of positive molecular replicates out of 8. The Cq value for each assay represents the number of cycles where the detection curve crossed the background fluorescence threshold. The starting copy number represents the number of DNA copies per microliter in the DNA extract.

Sample ID	EntTri Number of Positive Replicates	EntTri Cq	EntTri Starting Copy Number (copies/reaction)	PLamp Number of Positive Replicates	PLamp Cq	PLamp Starting Copy Number (copies/reaction)
21018	0	0	0	1	42.20	4.74
21047	1	39.09	1.23	0	0	0
21050	1	40.30	0.66	0	0	0

Discussion

In this study, we evaluated the use of eDNA sampling methods to detect Pacific Lamprey in several Puget Sound and coastal Washington streams and we were able to detect eDNA from Pacific Lamprey in three separate watersheds. However, it is important to place eDNA detections from Pacific Lamprey in the proper context so that data are interpreted correctly. A positive

eDNA detection indicates that DNA from Pacific Lamprey was collected on a filter and detected in the lab. Environmental DNA data cannot tell us which life stage produced the DNA that was detected or whether the streams sampled contained spawning and rearing habitat or were simply habitats being used by adults. Additionally, there are several vectors besides live fish than can contribute DNA to the environment including carcasses, predators, sampling gear, and boat traffic (Merkes et al. 2014; Kamoroff and Goldberg 2018). It is also important to note that a lack of eDNA detections should not be interpreted as evidence that Pacific Lamprey are not present in these streams. eDNA detections are influenced by a number of biotic and abiotic factors (Barnes and Turner 2016). If samples in this study were collected in sparsely inhabited streams, too far away from occupied habitat, or at a time of year when eDNA was difficult to detect due to limited eDNA shedding or sub-optimal environmental conditions, false negatives (lack of eDNA detection when the species is present in the stream) may occur. To decrease the probability of false negatives, replicate eDNA samples (i.e., water filters) should be collected at each site, but replicate samples were not collected in this study.

Although we were able to detect Pacific Lamprey DNA in multiple streams, our overall detection rate was relatively low. Only 3 of 70 samples were positive (approximately four percent), and the positive samples only had a single positive molecular replicate out of eight total. There are several possible explanations for the low detection rate we observed in this study. One explanation is that most of the streams we surveyed were uninhabited by Pacific Lamprey or had very low abundances. The markers used in this study were very sensitive at detecting Pacific Lamprey eDNA (Carim et al. 2017; Ostberg et al. 2019; Hannah and DeHaan 2023), and we assume that we would detect Pacific Lamprey eDNA if it was present in the environment. Previous surveys of Pacific Lamprey eDNA in Western Washington watersheds showed that lamprey were consistently detected, but many of the streams surveyed were larger than the ones in this study (Ostberg et al. 2018, 2019).

Environmental DNA detection rates often change over time and are influenced by species movement patterns, life history traits, and environmental conditions (Barnes et al. 2014; Rees et al. 2014; Barnes and Turner 2016; Mize et al. 2019). The samples collected in this study were all taken at a single point in time in late summer when flows were lowest relative to other times of the year, and the entire study area was in a drought. In a previous study of Pacific Lamprey eDNA detections in Puget Sound watersheds, Ostberg et al. (2018) observed higher detection rates during spring eDNA sampling events compared to fall sampling. The authors partially attributed this difference in detections to seasonal flow differences, where higher flows in the fall may have diluted the eDNA signal in several streams. Temporal replicate sampling in Coastal Washington streams with known Pacific Lamprey occupancy could help to determine the optimal sampling strategy in the future.

Pacific Lamprey have a unique life history where juveniles spend four to six years burrowed in the substrate before emerging and migrating to the anadromous environment and returning to freshwater streams as adults to spawn (Close et al. 2002), and these life history traits should be considered when interpreting our results and planning future studies. The seasonal life history differences may allow for Pacific Lamprey eDNA to be more readily detected during spring, when DNA from spawning and decomposing adults and migrating larvae is more readily present in the water column (Ostberg et al. 2018). If only ammocoetes of Pacific Lamprey were

present in late summer, being burrowed in sediments may have inhibited their detection in water column samples, and there is likely a more appropriate eDNA sediment sampling method for detecting ammocoetes (Olmstead 2019; Baltazar-Soares et al. 2022). A study looking at Pacific Lamprey eDNA in summertime which sampled both the water column and sediment of a river flowing into Puget Sound did not detect Pacific Lamprey eDNA in the water column but did have positive detections in the sediment (Brown 2019; Olmstead 2019). The authors noted that sediment sampling for Pacific Lamprey eDNA may be a better method than water sampling to detect larval lamprey presence year-round (Brown 2019; Olmstead 2019). Moreover, it is likely that sampling the water column for eDNA in this study was conducted outside the optimal sampling period for Pacific Lamprey detections in water samples. Temporal replication of water column and sediment sampling in streams with known Pacific Lamprey occupancy could help to determine the optimal sampling strategy in the future.

To summarize, it's important to recognize that eDNA detections for Pacific Lamprey in this study should not be interpreted as confirmation that a population of live fish are present (especially given the low detection rates observed here), and at the same time, the lack of detections should not be interpreted to mean the species does not occupy these streams. The markers and sampling techniques used in this study have been employed in other watersheds to detect Pacific Lamprey eDNA, and it appears there was little Pacific Lamprey eDNA present in the water column of streams we sampled during the timeframe of this study. The low amount of Pacific Lamprey eDNA present in our samples highlights the benefit of using two markers that may detect different genetic material to improve detection at low abundance. Importantly, eDNA is best used in conjunction with other physical sampling techniques, and the combination of multiple sampling approaches (e.g., eDNA, electrofishing, etc.) should be used to determine the status of Pacific Lamprey in these streams.

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References

Baltazar-Soares, M., A. C. Pinder, A. J. Harrison, W. Oliver, J. Picken, J. R. Britton, and D. Andreou. 2022. A noninvasive eDNA tool for detecting sea lamprey larvae in river sediments: Analytical validation and field testing in a low-abundance ecosystem. Journal of Fish Biology 100:1455-1463.

Barnes, M. A., and C. R. Turner. 2016. The ecology of environmental DNA and implications for conservation genetics. Conservation Genetics 17(1):1–17.

Barnes, M. A., C. R. Turner, C. L. Jerde, M. A. Renshaw, W. L. Chadderton, and D. M. Lodge. 2014. Environmental Conditions Influence eDNA Persistence in Aquatic Systems. Environmental Science & Technology 48(3):1819–1827.

Brown, S. 2019. Environmental DNA monitoring for Pacific, River and Western Brook Lamprey from the Nisqually River. Washington Department of Fish and Wildlife, Olympia.

Carim, K. J., J. C. Dysthe, M. K. Young, K. S. McKelvey, and M. K. Schwartz. 2017. A Noninvasive Tool to Assess the Distribution of Pacific Lamprey (*Entosphenus tridentatus*) in the Columbia River Basin. PLoS One 12(1):e0169334.

Close, D. A., M. S. Fitzpatrick, and H. W. Li. 2002. The Ecological and Cultural Importance of a Species at Risk of Extinction, Pacific Lamprey. Fisheries 27(7):19–25.

Goldberg, C. S., D. S. Pilliod, R. S. Arkle, and L. P. Waits. 2011. Molecular Detection of Vertebrates in Stream Water: A Demonstration Using Rocky Mountain Tailed Frogs and Idaho Giant Salamanders. PLOS ONE 6(7):e22746.

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, M. B. Laramie, A. R. Mahon, R. F. Lance, D. S. Pilliod, K. M. Strickler, L. P. Waits, A. K. Fremier, T. Takahara, J. E. Herder, and P. Taberlet. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolution 7(11):1299–1307.

Hannah, A. P., and P. W. DeHaan. 2023. Pacific Lamprey Environmental DNA Maker Lab Validation: Final Report. Page 8. USFWS, Onalaska, WI.

Kamoroff, C., and C. S. Goldberg. 2018. An issue of life or death: using eDNA to detect viable individuals in wilderness restoration. Freshwater Science 37(3):685–696.

Luzier, C. W., H. Schaller, J. Brostrom, C. Cook-Tabor, D. H. Goodman, R. D. Nelle, K. G. Ostrand, and B. Streif. 2011. Pacific Lamprey (Entosphenus tridentatus) assessment and template for conservation measures. Page 282. USFWS, Portland, OR.

Merkes, C. M., S. G. McCalla, N. R. Jensen, M. P. Gaikowski, and J. J. Amberg. 2014. Persistence of DNA in Carcasses, Slime and Avian Feces May Affect Interpretation of Environmental DNA Data. PLOS ONE 9(11):e113346.

Mize, E. L., R. A. Erickson, C. M. Merkes, N. Berndt, K. Bockrath, J. Credico, N. Grueneis, J. Merry, K. Mosel, M. Tuttle-Lau, K. V. Ruden, Z. Woiak, J. J. Amberg, K. Baerwaldt, S. Finney,

and E. Monroe. 2019. Refinement of eDNA as an early monitoring tool at the landscape-level: study design considerations. Ecological Applications 29(6):UNSP e01951.

Olmstead, J. J. 2019. Environmental DNA (eDNA) sediment sampling: a method for detecting larval lampreys in riverine habitat. Master's thesis. The Evergreen State University, Olympia, Washington.

Ostberg, C. O., D. M. Chase, M. C. Hayes, and J. J. Duda. 2018. Distribution and seasonal differences in Pacific Lamprey and Lampetra spp eDNA across 18 Puget Sound watersheds. Peerj 6:e4496.

Ostberg, C. O., D. M. Chase, M. S. Hoy, J. J. Duda, M. C. Hayes, J. C. Jolley, G. S. Silver, and C. Cook-Tabor. 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 1(2):131–143.

Plumb, M. 2019. Pacific lamprey 2019 regional implementation plan for the Washington coast/Puget Sound regional management units. Pacific Lamprey Conservation Initiative, Conservation Team.

Rees, H. C., B. C. Maddison, D. J. Middleditch, J. R. M. Patmore, and K. C. Gough. 2014. REVIEW The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. Journal of Applied Ecology 51(5):1450–1459.

Reid, S. B., and D. H. Goodman. 2015. Detectability of Pacific Lamprey Occupancy in Western Drainages: Implications for Distribution Surveys. Transactions of the American Fisheries Society 144(2):315–322.

Schmelzle, M. C., and A. P. Kinziger. 2016. Using occupancy modelling to compare environmental DNA to traditional field methods for regional-scale monitoring of an endangered aquatic species. Molecular Ecology Resources 16(4):895–908.

Smith, M. M., and C. S. Goldberg. 2020. Occupancy in dynamic systems: accounting for multiple scales and false positives using environmental DNA to inform monitoring. Ecography 43(3):376–386.

Sutter, M., and A. P. Kinziger. 2019. Rangewide tidewater goby occupancy survey using environmental DNA. Conservation Genetics 20(3):597–613.

USFWS. 2004. Endangered and threatened-wildlife and plants; 90-day finding on a petition to list three species of lampreys as threatened or endangered. Federal Register 69:77158–77167.

Appendices

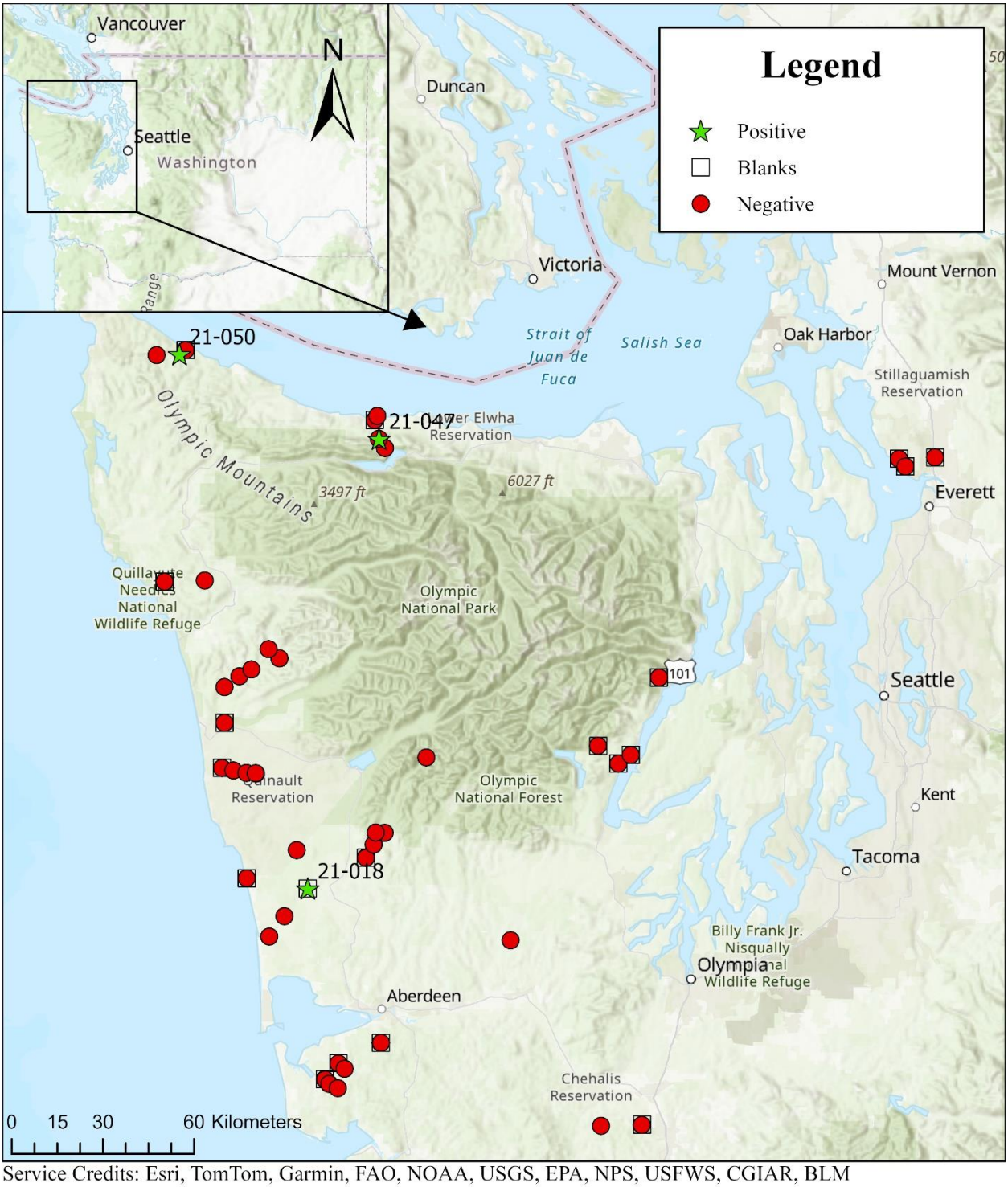
Appendix A. eDNA detection data for Coastal Washington and Puget Sound streams surveyed in this study.

Sample	Collection Date	Location	Number Filters Used	EntTri Number Positive Replicates	EntTri Mean Cq Value	PLamp Number Positive Replicates	PLamp Mean Cq Value	Notes
21001	07/29/21	East Fork Satsop River	1	0	0.00	0	0.00	
21002	08/02/21	Lincoln Creek	1	0	0.00	0	0.00	Field Blank
21003	08/02/21	Lincoln Creek	2	0	0.00	0	0.00	
21004	08/02/21	Lincoln Creek	2	0	0.00	0	0.00	
21005	08/03/21	Lilliwaup Creek	1	0	0.00	0	0.00	Field Blank
21006	08/03/21	Lilliwaup Creek	1	0	0.00	0	0.00	
21007	08/03/21	Eagle Creek	1	0	0.00	0	0.00	Field Blank
21008	08/03/21	Eagle Creek	1	0	0.00	0	0.00	
21010	08/17/21	Newskah Creek	1	0	0.00	0	0.00	Field Blank
21011	08/17/21	Newskah Creek	1	0	0.00	0	0.00	Field Blank
21012	08/17/21	Newskah Creek	1	0	0.00	0	0.00	
21013	08/17/21	Newskah Creek	1	0	0.00	0	0.00	
21014	08/17/21	Johns River	1	0	0.00	0	0.00	Field Blank
21015	08/17/21	Johns River	1	0	0.00	0	0.00	
21016	08/17/21	Johns River	1	0	0.00	0	0.00	
21017	08/25/21	Copalis River	1	0	0.00	0	0.00	Field Blank
21018	08/25/21	Copalis River	1	0	0.00	1	42.20	
21019	08/25/21	Cedar Creek	1	0	0.00	0	0.00	
21020	08/25/21	Copalis River	2	0	0.00	0	0.00	
21021	08/25/21	Moclips River	1	0	0.00	0	0.00	Field Blank
21022	08/25/21	Moclips River	2	0	0.00	0	0.00	
21023	08/25/21	North Fork Moclips River	2	0	0.00	0	0.00	
21024	08/26/21	WF Humptulips River	1	0	0.00	0	0.00	Field Blank

Sample	Collection Date	Location	Number Filters Used	EntTri Number Positive Replicates	EntTri Mean Cq Value	PLamp Number Positive Replicates	PLamp Mean Cq Value	Notes
21025	08/26/21	WF Humptulips River	1	0	0.00	0	0.00	
21026	08/26/21	WF Humptulips River	1	0	0.00	0	0.00	
21027	08/26/21	WF Humptulips River	1	0	0.00	0	0.00	
21028	08/26/21	Donkey Creek	1	0	0.00	0	0.00	
21029	08/26/21	WF Humptulips River	1	0	0.00	0	0.00	
21030	08/26/21	WF Humptulips River	1	0	0.00	0	0.00	
21031	09/07/21	Fulton Creek	1	0	0.00	0	0.00	Field Blank
21032	09/07/21	Fulton Creek	1	0	0.00	0	0.00	
21033	09/07/21	Lilliwaup Creek	1	0	0.00	0	0.00	Field Blank
21034	09/07/21	Lilliwaup Creek	1	0	0.00	0	0.00	
21035	09/08/21	Clearwater River	1	0	0.00	0	0.00	Field Blank
21036	09/08/21	Clearwater River	1	0	0.00	0	0.00	
21037	09/08/21	Clearwater River	1	0	0.00	0	0.00	
21038	09/08/21	Clearwater River	1	0	0.00	0	0.00	
21039	09/08/21	Clearwater River	1	0	0.00	0	0.00	
21040	09/08/21	Clearwater River	1	0	0.00	0	0.00	
21041	09/08/21	Snahapish River	1	0	0.00	0	0.00	
21042	09/09/21	Lyre River	1	0	0.00	0	0.00	Field Blank
21043	09/09/21	Lyre River	1	0	0.00	0	0.00	
21044	09/09/21	Lyre River	1	0	0.00	0	0.00	
21045	09/09/21	Lyre River	1	0	0.00	0	0.00	

Sample	Collection Date	Location	Number Filters Used	EntTri Number Positive Replicates	EntTri Mean Cq Value	PLamp Number Positive Replicates	PLamp Mean Cq Value	Notes
21046	09/09/21	Lyre River	1	0	0.00	0	0.00	
21047	09/09/21	Lyre River	1	1	39.09	0	0.00	
21048	09/10/21	Sekiu River	1	0	0.00	0	0.00	Field Blank
21049	09/10/21	Sekiu River	1	0	0.00	0	0.00	
21050	09/10/21	Sekiu River	1	1	40.30	0	0.00	
21051	09/10/21	Sekiu River	1	0	0.00	0	0.00	
21052	09/15/21	Quilceda Creek	1	0	0.00	0	0.00	Field Blank
21053	09/15/21	Quilceda Creek	1	0	0.00	0	0.00	
21054	09/15/21	Tulalip Creek	1	0	0.00	0	0.00	Field Blank
21055	09/15/21	Tulalip Creek	1	0	0.00	0	0.00	
21056	09/15/21	Mission Creek	1	0	0.00	0	0.00	Field Blank
21057	09/15/21	Mission Creek	1	0	0.00	0	0.00	
21058	09/22/21	Raft river	1	0	0.00	0	0.00	Field Blank
21059	09/22/21	Raft river	2	0	0.00	0	0.00	
21060	09/22/21	Raft river	2	0	0.00	0	0.00	
21061	09/22/21	Raft river	2	0	0.00	0	0.00	
21062	09/22/21	Raft river	1	0	0.00	0	0.00	
21063	09/22/21	Raft river	1	0	0.00	0	0.00	
21064	09/23/21	Goodman Creek	1	0	0.00	0	0.00	Field Blank
21065	09/23/21	Goodman Creek	1	0	0.00	0	0.00	
21066	09/23/21	Goodman Creek	1	0	0.00	0	0.00	
21067	09/28/21	Elk River	1	0	0.00	0	0.00	Field Blank
21068	09/28/21	Elk River	3	0	0.00	0	0.00	
21069	09/28/21	Elk River	3	0	0.00	0	0.00	
21070	09/28/21	Elk River	3	0	0.00	0	0.00	
21071	09/28/21	Elk River	3	0	0.00	0	0.00	

Appendix B. Map of sampling locations and results of project. The colors and shapes of points denote the results of eDNA analysis. Green stars represent sample locations with a positive eDNA detection, red circles represent sample locations with no positive detections, and empty squares show locations where field blanks were taken.



Appendix C. qPCR Master mix concentrations for the two Pacific Lamprey eDNA assays used in this study.

Reagent	**Volume (µl)**	**Final Concentration**
2X Taq Man Environmental Master Mix	10	1X
Forward Primer	1	0.5 µM
Reverse Primer	1	0.5 µM
Probe	1	0.125 µM
H2O	3	
Template DNA	4	
TOTAL VOLUME	**20**	