

U.S. Fish and Wildlife Service

Using eDNA Sampling to Detect Pacific Lamprey in a Large River: 2016 Wenatchee River Pilot Study



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On the cover: MCFWCO technician Mark Inc collects an eDNA sample in the Lower Wenatchee River. USFWS photograph by Ann Grote.

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2016 WENATCHEE RIVER PILOT STUDY**

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Abstract- Although Pacific Lampreys were historically abundant in the Pacific Northwest, runs have declined dramatically as a result of barriers to upstream passage, juvenile entrainment, habitat loss, and compromised water quality. Defining the current distribution of Pacific Lamprey is a major component of the Pacific Lamprey Conservation Initiative and is central to lamprey recovery efforts. Environmental DNA (eDNA) sampling may be a time-saving and cost-effective method to broadly assess habitat for Pacific Lamprey presence. However, aquatic macrofaunal eDNA methods are relatively new, with many studies occurring in small streams, and focusing on free-swimming fishes. This pilot study expanded the application of eDNA sampling methods to a benthically-oriented species in a large river system. We evaluated the capacity of eDNA sampling to detect Pacific Lamprey presence in the Wenatchee River, Washington in June 2016. The initial results of this study are promising: Pacific Lamprey DNA was detected at all ($n = 5$) Lower Wenatchee River sites located within the known distribution of the species. Pacific Lamprey DNA was also detected at four sites in the Upper Wenatchee River near locations where adult Pacific Lampreys had been re-introduced three months earlier. Pacific Lamprey DNA concentrations from paired-transect samples were similar (within an order of magnitude), suggesting that single samples at one river bank may be sufficient to document lamprey presence at a given location. These results suggest that eDNA sampling can be a valuable tool for evaluating Pacific Lamprey presence. However, because questions remain about detection probabilities when the target species is extremely rare, we recommend that eDNA methods be combined with traditional sampling methods, especially in systems where little is known about Pacific Lamprey distribution.

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Table of Contents

List of Tables	iii
List of Figures	iii
Introduction.....	1
Study Area and Background	2
Methods.....	4
Results.....	6
Discussion.....	8
Acknowledgments.....	12
Literature Cited	12

List of Tables

Table 1. Sampling locations and Pacific Lamprey DNA concentrations (DNA copies/L) in the Wenatchee River subbasin in June 2016.....	6
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List of Figures

Figure 1: Release locations of adult Pacific Lampreys translocated by the Yakama Nation Fisheries Program in spring 2016. Prior to this translocation, Pacific Lampreys were not present in the Wenatchee River upstream of Tumwater Dam.....	3
Figure 2: Upper and Lower Wenatchee River eDNA survey locations sampled in June 2016.....	5
Figure 3: Pacific Lamprey DNA detections by sample location in the Wenatchee River in June 2016. DNA concentrations (DNA copies/L) for the transect sites are pooled to include the number of copies detected at both river banks.....	7

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Introduction

Defining the current distribution of Pacific Lamprey *Entosphenus tridentatus* is a major component of the Pacific Lamprey Conservation Initiative and is central to species recovery efforts (Luzier et al. 2011). Lamprey presence and distribution is typically assessed in small streams with electrofishing surveys targeting ammocoetes (Dunham et al. 2013, Reid and Goodman 2015) and spawning surveys targeting adults or nests (Mayfield et al. 2014). While effective, these traditional sampling methods can be time- and resource-intensive, and are difficult to implement in large streams and rivers (Jolley et al. 2012). Detection methods that can scale from small streams to large rivers would be valuable for rapidly evaluating systems where little or no lamprey information is available.

This pilot study investigated whether environmental DNA (eDNA) sampling could be used to detect Pacific Lamprey in large river systems and at low animal densities. Environmental DNA is genetic material shed into the surrounding environment from sources such as tissues, feces, scales, or gametes of an animal. This DNA can be captured and analyzed to determine the presence of a target species without physically handling any specimens (Jerde et al. 2011, Turner et al. 2014). Research has shown that eDNA sampling can be more sensitive, efficient, and cost effective than traditional sampling methods (Dejean et al. 2012, McKelvey et al. 2016, Thomsen et al. 2012). Environmental DNA sampling has been used to detect endangered fish species (Janosik and Johnson 2015) and to describe the distribution of salmonids in freshwater systems (Laramie et al. 2015, McKelvey et al. 2016). However eDNA methods are relatively new, and much of the work in lotic systems has been in small streams (Jane et al. 2015, McKelvey et al. 2016, Wilcox et al. 2013) and focused on free-swimming fishes (Jerde et al. 2011, Bergman et al. 2016). The efficacy of this approach to accurately detect benthically-oriented animals such as lampreys that are often found in large river systems remains largely untested.

Initial research provides some insights into the efficacy of eDNA sampling for detecting lampreys. For example, research on Sea Lamprey *Petromyzon marinus* suggests that adult lampreys may be more readily detected than ammocoetes, particularly during spawning (Gustavson et al. 2015, Gingera et al. 2016). Similar to other fluvial species (Thomsen et al. 2012, Pilliod et al. 2013), lamprey detectability is related to animal density. Initial work with Sea Lamprey ammocoetes suggest that eDNA detection rates are low at low and even medium larval densities, whereas at high densities larvae appear highly detectable. (Gingera et al. 2016). However, research has not tested whether there are differences in detectability between different life stages of lampreys. In particular, detection of lampreys may be more difficult than for other fish because there are times of year when only the substrate-dwelling ammocoetes are present, and much of their shed DNA may not be completely mixed in the water column. Furthermore, eDNA detection efficiencies may be lower in larger rivers where large water volumes may dilute DNA concentrations, particularly when animals are present in low abundance. Together, these

unknowns warrant further study into eDNA sampling for detection of Pacific Lampreys and similar species living in larger lotic systems.

Study Area and Background

Beginning in 2009, the Mid-Columbia Fish and Wildlife Conservation Office (MCFWCO) collected information on Pacific Lamprey distribution in the Wenatchee River. The results from repeated random (occupancy) and non-random (targeted) electrofishing surveys indicated that Pacific lampreys occupied the Wenatchee River up to rkm 48.6, approximately 1 rkm downstream of Tumwater Dam (Barb Kelly-Ringel, USFWS, unpublished data). Despite historical accounts of Pacific Lampreys throughout the Wenatchee River sub-basin, recent surveys found no evidence of them upstream of Tumwater Dam at rkm 49.6 (Johnsen and Nelson 2012, Ann Grote, USFWS, unpublished data). These results suggest that Tumwater Dam or current fishway operations impede Pacific Lamprey passage. For the purposes of this document “Upper River” refers to the main stem Wenatchee River upstream of Tumwater Dam, and “Lower River” refers to the main stem Wenatchee River downstream of the dam.

In 2016, Pacific Lamprey distribution in the Wenatchee River changed when the Yakama Nation Fisheries Program (YNFP) began an adult lamprey translocation program intended to supplement the existing run downstream of Tumwater Dam and re-introduce natural reproduction upstream of Tumwater Dam. All translocated Pacific Lampreys were originally collected at hydroelectric dams in the lower Columbia River (Bonneville, The Dalles, and John Day dams), and were held at the YNFP Prosser Hatchery prior to release. On March 17, 2016, 180 PIT-tagged translocated adult Pacific Lampreys were released in the Wenatchee River (Ralph Lampman, YNFP, pers. comm.). Eighty adults were released in the Lower River: 50 near the Wenatchee City limits and 30 approximately 1 km downstream of Tumwater Dam (Figure 1). One hundred adults were released in the Upper River: 50 at the upstream end of the Tumwater Dam impoundment (Lake Jolanda) and 50 downstream of the confluence of Chiwaukum Creek (Figure 1). On May 3, 2016, 30 additional translocated adult Pacific Lampreys were released into the Tumwater Dam fish ladder (Figure 1). As a result of these translocations, the only Pacific Lampreys present in the Upper River during spring 2016 were translocated adults. At the time of eDNA sampling in mid-June, it was thought that these adults had migrated near spawning locations but had not yet spawned. During this same time, translocated adults, volitional adult migrants, and naturally reproduced juveniles were distributed throughout the Lower River.

The objectives of this study were to: 1) determine if eDNA sampling could detect Pacific Lamprey presence in a large river system, 2) investigate whether detections were consistent with the known Pacific Lamprey distribution determined by traditional methods, and, 3) compare detections and eDNA concentrations from low-density and high-density reaches and in paired samples spanning the river.

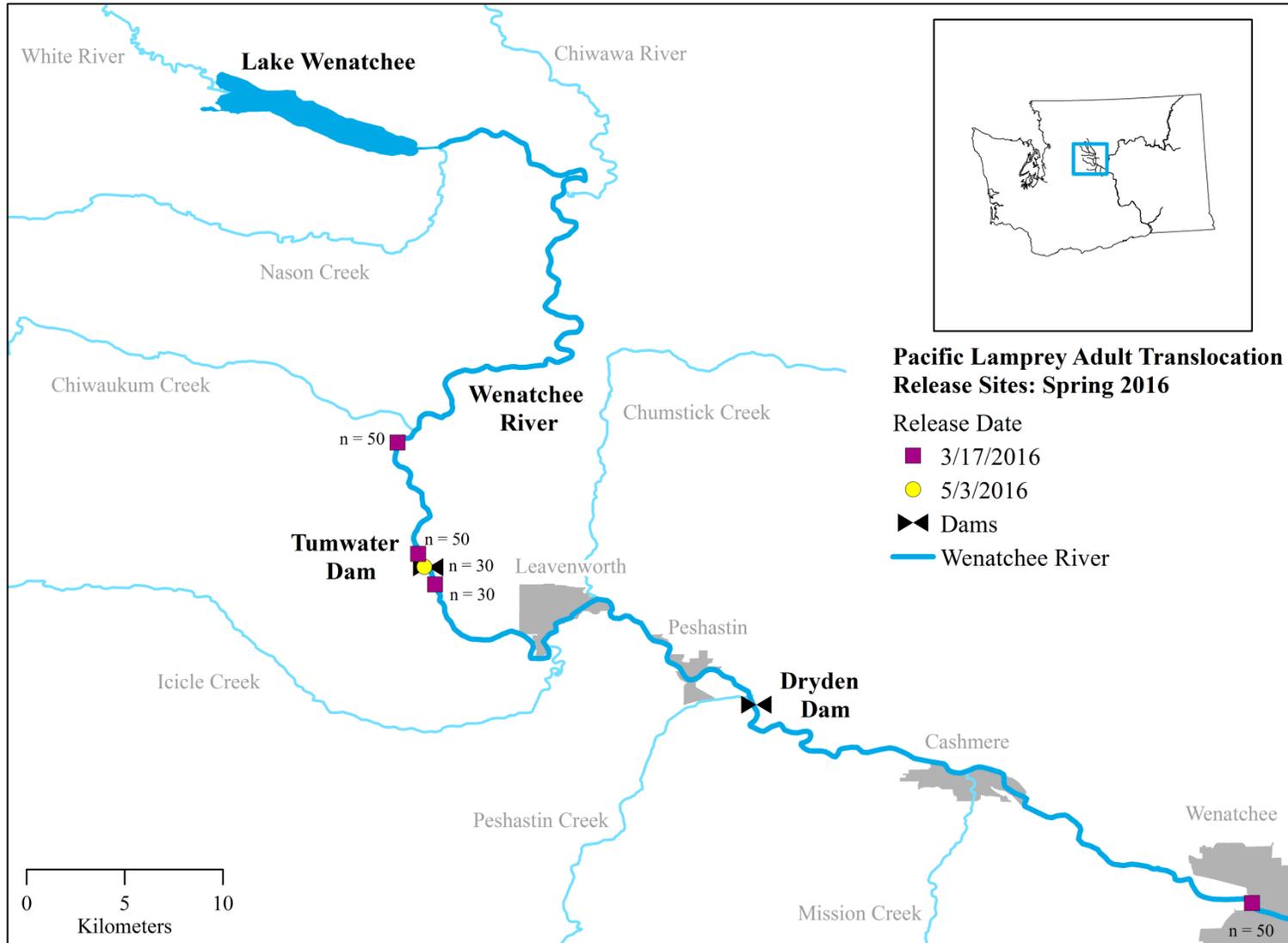


Figure 1: Release locations of adult Pacific Lampreys translocated by the Yakama Nation Fisheries Program in spring 2016. Prior to this translocation, Pacific Lampreys were not present in the Wenatchee River upstream of Tumwater Dam.

Methods

Two-person crews collected eDNA samples by filtering 5-L samples through 1.5- μ m pore size glass microfiber filters at collection sites following standard protocols (Carim et al. 2016a). Samples were collected from 13–21 June 2016. Sample locations were chosen for ease of access (road-based), typically at bridges where both sides of the river were accessible (Figure 2). In the Upper River, the two lowest transects (UWEN01 and UWEN02) were immediately upstream of the sites where adult translocated Pacific Lampreys had been released three months earlier. Samples were returned to the NGC for processing on June 24, 2016.

Samples were stored at -20 °C until analyzed. DNA extraction was performed on half of the sample filter using the Qiagen DNEasy® Blood and Tissue Kit with a modified protocol (Carim et al. 2016b). The other half of the sample filter was retained and stored at -20 °C. All samples were analyzed for the presence of Pacific Lamprey DNA using a quantitative PCR (qPCR) assay developed in the NGC (Carim et al. 2017). Each sample was analyzed on a StepOne Plus Real-time PCR Instrument (Life Technologies) instrument in triplicate. A sample was considered positive for the presence of the Pacific Lamprey if at least one of the three reactions amplified DNA of the target species.

To quantify DNA, all samples were run alongside a five-level standard curve dilution series (6 250, 1 250, 250, 50, and 10 DNA copies per 4 μ l). The total number of DNA copies was averaged across all positive reactions for a given sample. We then multiplied the average copies per reaction by 10 to obtain the average copies per liter of water. (DNA was extracted from half of the filter producing a 100 μ l elution volume, each reaction used 4 μ l of the elution. The long version of this calculation is as follows: (1) Multiply the average DNA quantity in the triplicate reaction by 25 to estimate the DNA quantity in 100 μ l volume of extracted DNA. (2) Multiply this number by 2 to estimate all DNA on one entire sample filter. (3) Divide this number by 5, which is the total number of liters sampled, to reach the estimated number of DNA copies per liter. All reactions included an internal positive control to ensure that it was effective and sensitive to the presence of Pacific Lamprey DNA, and to verify that DNA amplification was not inhibited. All laboratory experiments were conducted with negative controls to insure there was no contamination during DNA extraction or qPCR setup.

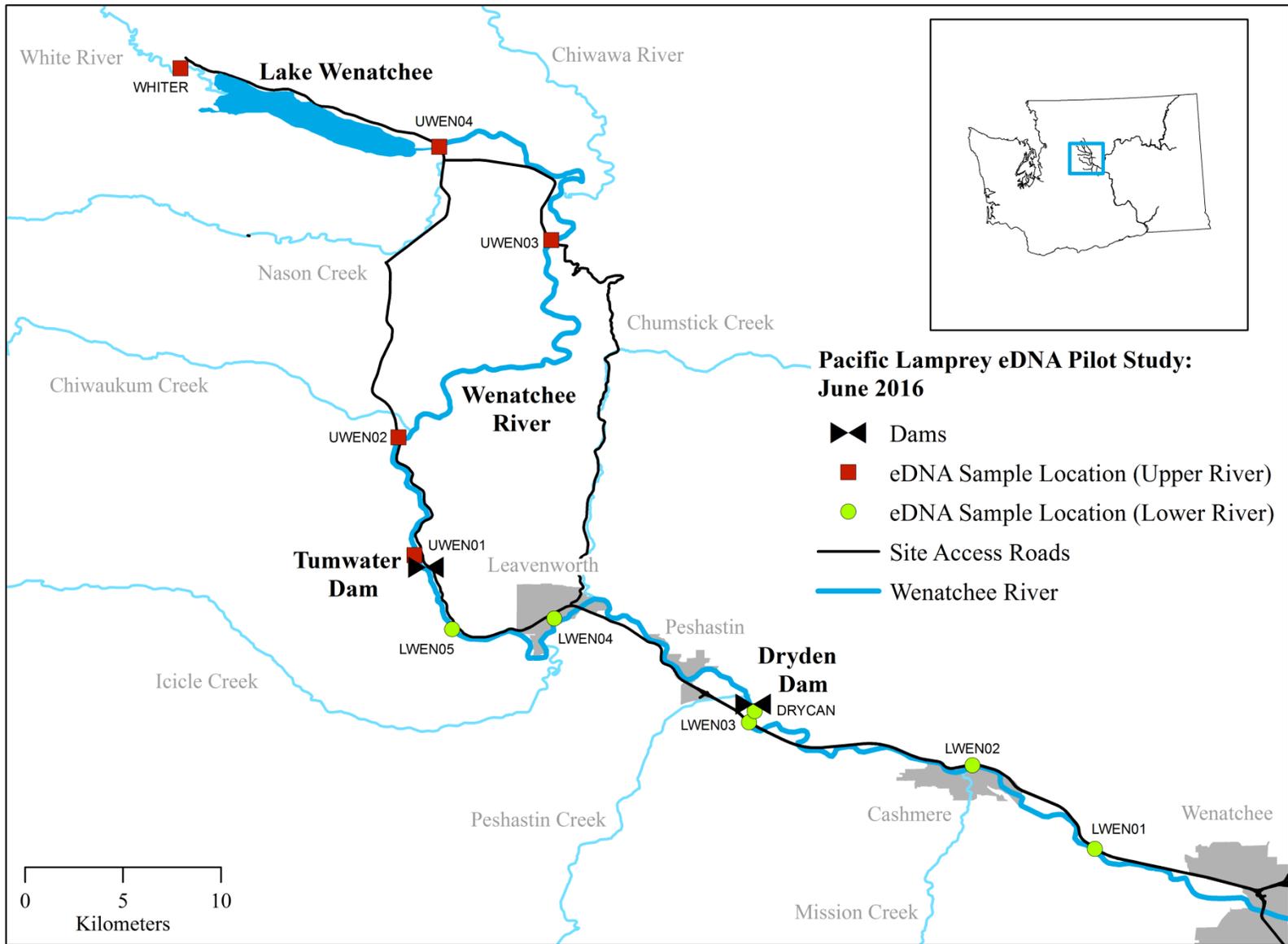


Figure 2: Upper and Lower Wenatchee River eDNA survey locations sampled in June 2016.

Results

No samples in this study showed evidence of PCR inhibition, and Pacific Lamprey DNA was not detected in any laboratory negative controls. Pacific Lamprey DNA was detected in 15 of the 20 Wenatchee River samples (Table 1). All five Lower River transects (LWEN01 – LWEN05) and the Dryden Canal were positive for Pacific Lamprey DNA. In addition, two of the Upper River Transects (UWEN01 and UWEN02) were also positive for Pacific Lamprey DNA. No Pacific Lamprey DNA was detected at the uppermost sample transects (UWEN03 and UWEN04), or in the White River. The number of positive PCR triplicates varied by site, from 0 – 3 (Table 1). Samples collected from the Lower River generally produced more positive reactions than those collected in the Upper River. Pacific Lamprey DNA was detected at both banks of the river in all transects that tested positive.

Table 1: Sampling locations and Pacific Lamprey DNA concentrations (DNA copies/L) in the Wenatchee River sub basin in June 2016.

Stream and Reach	Site	Date	Latitude (N)	Longitude (W)	# Positive Detections (PCR Triplicate)	Mean DNA copies/L (\pm SD)
Wenatchee River (downstream of Tumwater Dam)	LWEN01LB	6/13/16	47.48745	120.41376	3	132 (\pm 19)
	LWEN01RB	6/13/16	47.48715	120.41444	3	134 (\pm 24)
	LWEN02LB	6/14/16	47.52584	120.46994	3	68 (\pm 30)
	LWEN02RB	6/14/16	47.52504	120.47003	3	119 (\pm 6)
	LWEN03LB	6/14/16	47.54554	120.57269	3	62 (\pm 18)
	LWEN03RB	6/14/16	47.54612	120.57324	3	210 (\pm 70)
	LWEN04LB	6/14/16	47.59326	120.66212	3	41 (\pm 17)
	LWEN04RB	6/14/16	47.59319	120.66229	3	340 (\pm 68)
	LWEN05LB	6/15/16	47.58827	120.70908	2	5 (\pm 7)
	LWEN05RB	6/15/16	47.58802	120.70930	2	9 (\pm 10)
Wenatchee River (upstream of Tumwater Dam)	UWEN01LB	6/15/16	47.62299	120.72577	2	7 (\pm 7)
	UWEN01RB	6/15/16	47.62223	120.72639	2	11 (\pm 12)
	UWEN02LB	6/15/16	47.67585	120.73381	1	2 (\pm 4)
	UWEN02RB	6/15/16	47.67646	120.73376	3	14 (\pm 7)
	UWEN03LB	6/17/16	47.76669	120.66264	0	0
	UWEN03RB	6/17/16	47.76696	120.66344	0	0
	UWEN04LB	6/17/16	47.81015	120.71546	0	0
UWEN04RB	6/17/16	47.80973	120.71503	0	0	
Dryden Canal	DRYCAN	6/21/16	47.55069	120.57010	3	106 (\pm 52)
White River	WHITER01	6/17/16	47.84580	120.83384	0	0

Mean sample concentrations of DNA were 2–340 copies/L (Table 1). At each main stem river transect, DNA concentrations were similar (within the same order of magnitude) for samples drawn from each river bank. DNA concentrations from two transects, LWEN04 and UWEN02, varied by up to an eightfold difference between the right and left banks, but the remaining transect concentrations were more consistent. DNA concentrations were generally greater in the Lower River than the Upper River; the exception was transect LWEN05, where DNA concentrations were low and more similar to samples from the Upper River (Figure 3).

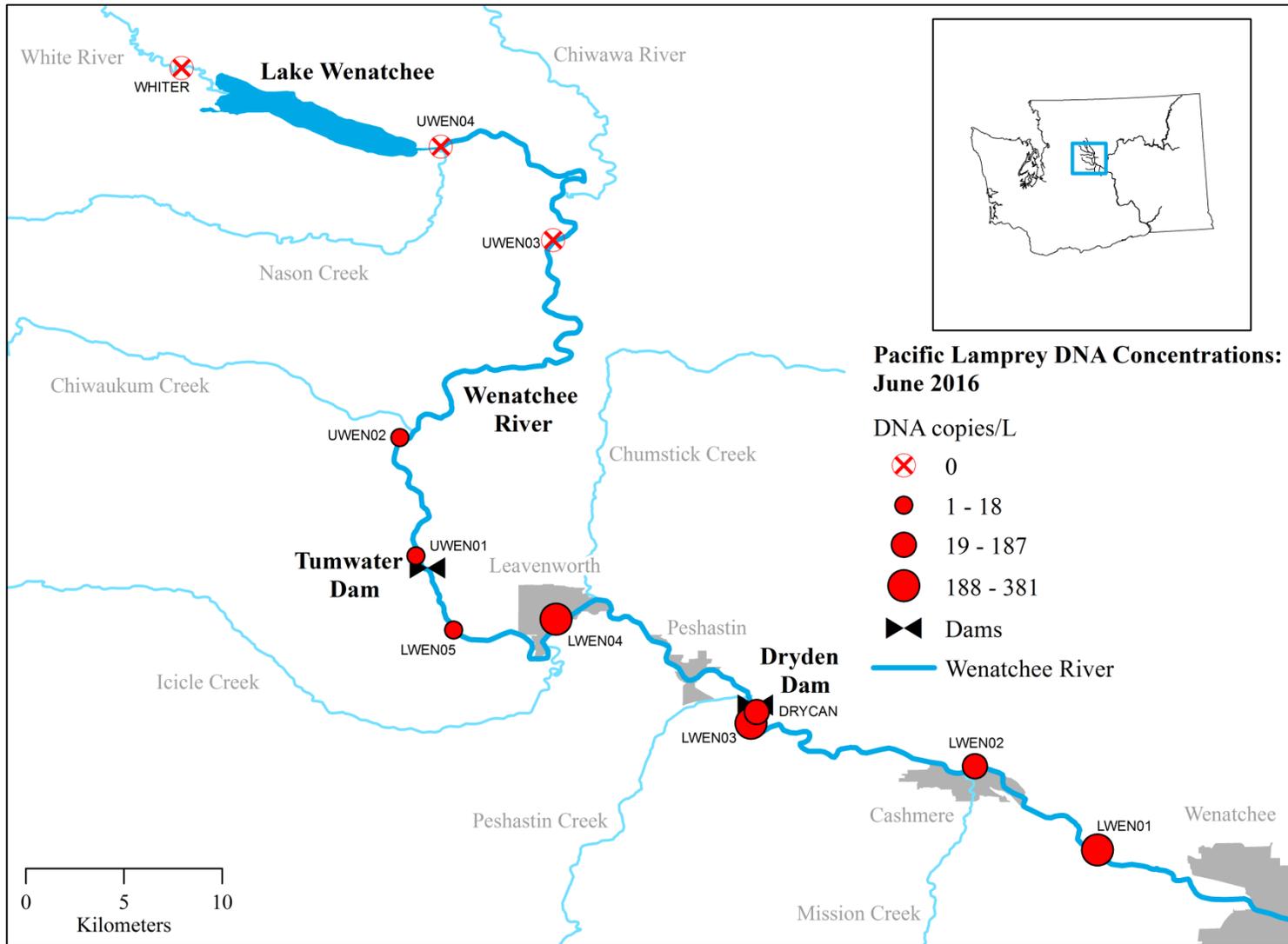


Figure 3: Pacific Lamprey DNA detections by sample location in the Wenatchee River in June 2016. DNA concentrations (mean copies/L) for the transect sites are pooled to include the number of copies detected at both river banks.

Discussion

This pilot study investigated the capacity for eDNA techniques to detect Pacific Lamprey presence within a large river system, and to corroborate the known Pacific Lamprey distribution in the main stem Wenatchee River. Taking into account the limitations of this pilot study (small sample size, lack of replication, and unknown eDNA detection probability) the initial results are promising: Pacific Lamprey DNA was detected in water sampled from the Wenatchee River (Objective 1) at locations that were easily accessible. Moreover, lamprey DNA was detected at locations that matched the known distribution of Pacific Lamprey (Objective 2). Observed DNA concentrations were generally greater in the Lower River (Objective 3) which was already occupied by lampreys prior to the YNFP adult translocation program.

This pilot study establishes that eDNA methods are capable of detecting Pacific Lampreys in a large river system, and validating the known distribution when conducted in a road-based, non-randomized sampling framework. This style of “convenience” sampling is attractive as minimal sampling effort and relatively few samples are required to provide a basic assessment of presence and distribution. For fine-scale information on the extent of occupancy in the main stem river, or in tributaries, more intensive eDNA sampling at fixed intervals (every 1 – 3 rkm) would provide greater resolution. In our study, for example, there is a 17 rkm gap between the highest DNA-positive transect (UWEN02) and the lowest DNA-negative transect (UWEN03), and the upper extent of Pacific Lamprey distribution within that gap is unknown.

Beyond evaluating whether eDNA sampling would detect Pacific Lampreys, this study sought to investigate how DNA concentrations might vary across areas of differing animal densities. When discussing the concentrations, care needs to be taken in making comparison across sites where environmental (flow, temperature, water chemistry) and biological (life stage and the size and number of animals) covariates were not standardized (Goldberg et al. 2016). With that caveat in mind, our results are consistent with what is known about Pacific Lamprey numbers in the Wenatchee River. Generally, the greatest Pacific Lamprey DNA concentrations were recorded at Lower River sampling locations. These DNA-rich sites are located downstream of Tumwater Dam in reaches of the river that were electrofished extensively in 2012, and that were occupied by larval Pacific Lampreys at that time (Ann Grote, USFWS, unpublished data). The DNA collected at these Lower River sites was presumably a combination of material from both from larval and juvenile Pacific Lampreys, along with adults (translocated and volitional migrants) that were present in the Lower River. The exception to this trend was the LWEN05 transect, where DNA concentrations were the lowest observed in the pilot study. This transect is located 3.5 rkm downstream of Tumwater Dam, and 2.6 rkm downstream of the uppermost site (prior to translocation) that is occupied by Pacific Lampreys (Barb Kelly-Ringel, USFWS, pers. comm.). Ammocoete habitat is extremely limited in this reach, where electrofishing surveys have detected only a few sparsely-occupied patches. Given the low densities of ammocoetes and adults in this

reach relative to other areas of the Lower Wenatchee, it is not surprising the DNA quantities were also comparatively lower than other sampling transects.

Upstream of Tumwater Dam, low concentrations of Pacific Lamprey DNA were detected at UWEN01 and UWEN02, near locations where the YNFP had released translocated adults on March 17, 2016 (Figures 1 and 3). It is unclear if the DNA collected at these sites was from adult Pacific Lampreys that remained in the area, their carcasses, or spawning products. Although we attempted to conduct field sampling before spawning began (thereby limiting our detections upstream of the dam to adults only), water temperatures in the Upper River ranged from 8.5–12.0 °C during sample collection. Pacific Lamprey spawning in the Mid-Columbia tributaries is thought to occur at temps greater than 10.0° C; so it is possible that translocated fish in the Upper River were already spawning during our sampling period in June 2016. On August 23, 2016 YNFP staff encountered young-of-the-year larval lampreys during an electrofishing survey just downstream of the UWEN02LB site (Tyler Beals, YNFP, pers. comm.); thus it appears that translocated adults had successfully reproduced at some point earlier in the summer.

Results from this study inform sampling efforts for more efficient data collection in future studies. For example, DNA concentrations from both sites within each transect (i.e., both the left and right river banks) were relatively consistent (within an order of magnitude), and there were no cases where DNA was detected at one side of a transect, but not the other. With the exception of UWEN02 (see discussion below), the number of replicates testing positive for amplified Pacific Lamprey DNA was consistent from samples collected at both banks. In addition, the only cases where some of the triplicates failed to amplify occurred at extremely low DNA concentrations (2 – 11 DNA copies/L). These results suggest that basic DNA information (presence, general concentration, number of amplified replicates) is consistent between samples collected from a transect, in which case collecting two samples per transect may be redundant. Single bank samples may be preferable for future studies where access to both river banks is limited, where minimizing collection and processing costs is a priority, and where additional sites can be sampled for the same overall processing costs.

Although DNA concentrations were generally similar within transects, the results from LWEN04 and UWEN02 varied by up to a factor of eight between the river left and right banks. For LWEN04, this is likely because of fine-scale habitat variability within the transect. Unlike the other sampling locations, the stream geomorphology and habitat immediately upstream of LWEN04 varies dramatically between banks. This transect is located in a side channel where the river right bank (340 copies/L) is a depositional area composed of sand, silt, and organic deposits. The right bank is highly suitable for larval lampreys: our staff routinely collect ammocoetes from this site for outreach and education purposes. The river left bank (41 copies/L) is scoured and armored with large boulders, and offers much less soft substrate and prime lamprey habitat. The distance between these two sample sites is only 23 m, and they border the

same side channel, but the DNA concentrations appear to reflect fine-scale differences in habitat suitability and possibly Pacific Lamprey abundance. At UWEN02, the DNA concentrations at the left bank (2 copies/L) and right bank (14 copies/L) were both extremely low, and the density of adults was unknown. This small effective sample size may have impacted the relative DNA concentrations across the sites at the UWEN02 transect.

No Pacific Lamprey DNA was detected upstream of UWEN02, suggesting that the translocated adults had not migrated further upriver at the time of sampling, or had done so in small enough numbers to remain undetected at the sample locations. A single translocated adult Pacific Lamprey was detected at the White River PIT Array on June 7, 2016 and an eDNA sample was collected immediately downstream of the array (WhiteR01, Figure 3) 10 days later on June 17, 2016. This sample did not contain Pacific Lamprey DNA. It is unclear from the PIT data if the tagged lamprey remained on site or moved elsewhere. If it was absent at the time of sample collection, the DNA result would have confirmed a “true” negative result. If it was present, the lamprey may have been too distant from the sampling location for sufficient levels of DNA to be collected, thus leading to a “false” negative result. This discrepancy highlights some of the difficulties arising from eDNA interpretation when target animal densities are extremely low, even when additional (telemetry) data is available. As with the concentration data, care needs to be taken in making inferences from these results.

Many of the benefits of eDNA studies are well-documented, including elimination of handling stress of specimens (Bergman et al. 2016), improved detection efficiencies for rare and cryptic species (Jerde et al. 2011, Goldberg et al. 2013), and efficient coverage of broad geographic areas (McKelvey et al. 2016). An additional advantage of this approach, and one that is especially appealing to less well-funded projects and species like Pacific Lamprey, is the efficiency of shared samples collected as a part of other eDNA monitoring programs. Each eDNA sample filter collected for this study can be analyzed for multiple species, providing the qPCR assays have been developed (Kellie Carim, USFS, pers. comm). Samples collected as a part of a Bull Trout *Salvelinus confluentus* survey, for example, could also be evaluated for Brook Trout *S. fontinalis* and Pacific Lamprey, thereby further reducing data collection and some sample analysis costs. Analyzing a pre-existing collection of samples, or even a subsample of filters collected in a watershed of interest, could therefore be very cost-effective. Future sample analysis costs are likely to decrease, due to economies of scale and improvements to qPCR processing routines.

Although this study has provided information to better understand eDNA sampling for detection of Pacific Lampreys, there are still inherent limitations of this tool which can only be resolved with the companion use of traditional sampling methods. First, eDNA sampling cannot provide information on the age or size structure of a population. Because we were unable to determine if detections were from Pacific Lamprey ammocoetes or adults in the Upper Wenatchee River,

eDNA results were uninformative for confirming that Pacific Lamprey introductions lead to spawning. Second, the relationship between eDNA quantities and organism abundance is variable and can be influenced by downstream drift of DNA. Third, detection and estimates of eDNA quantity in a sample are less consistent when DNA quantity is low. In our study, this was particularly relevant when sampling areas with fine scale habitat variation. Our results suggest that to increase detection probabilities and DNA quantities, samples should be taken in areas with the most favorable habitat if fine scale habitat variation exists within a transect.

Additional research into the transport and persistence of DNA in larger river systems would provide insight into the minimum density and proximity of individuals needed to generate detectable levels of eDNA in the system (e.g., Jane et al. 2014, Wilcox et al. 2016). Well-planned sampling strategies may be able to provide insight into addressing some of these limitations. For example, sampling at regular spatial intervals can help determine areas of higher fish density, as those areas will have relatively more DNA compared to other sites. Collecting eDNA samples in a particular location at regular time intervals throughout the year may provide insight into life history events. For example, we would expect to see increased quantities of eDNA during aggregation and spawning (e.g., Laramie et al. 2015) and likely upon emergence of ammocoetes from the sediment.

Despite these limitations, this study found that eDNA sampling can produce valuable information on the presence and distribution of Pacific Lampreys, even in large river systems and using convenience-based sampling. Given the limitations and unknowns of eDNA sampling in larger river systems, we suggest that eDNA sampling be replicated, and where possible, paired with additional sampling methods (electrofishing, redd surveys, or telemetry). Sampling redundancy (eDNA or traditional methods) is especially important for systems lacking information on current Pacific Lamprey distribution. For systems where there is prior information on Pacific Lamprey presence, replicated stand-alone eDNA sampling may be appropriate. With continued research to understand the applications of this tool, we are hopeful that eDNA sampling will prove to be an efficient, sensitivity, accurate and cost-effective option for conservation managers studying cryptic animals in larger river systems.

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