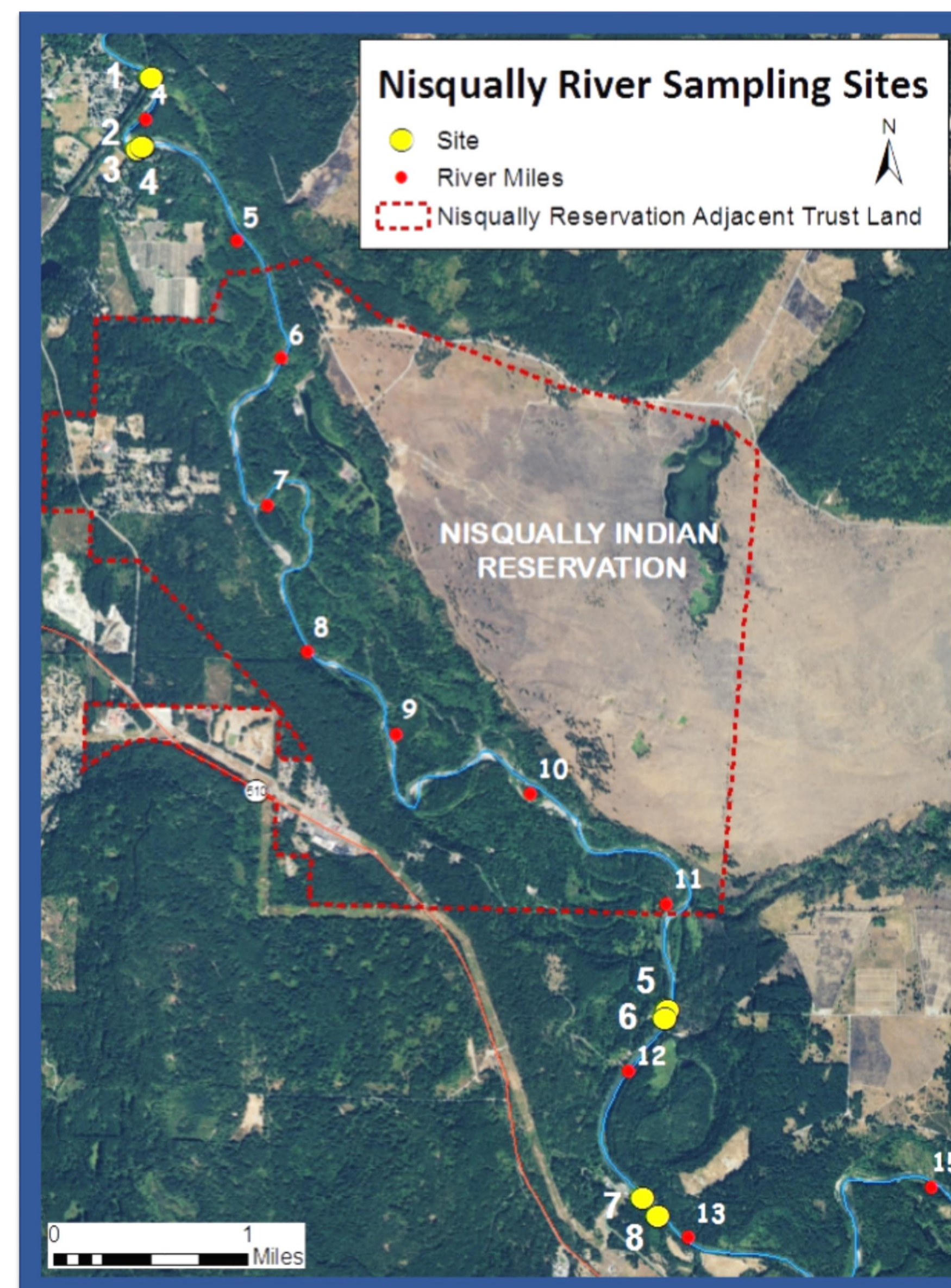


Using Environmental DNA (eDNA) sediment sampling to evaluate a detection method for Pacific Lamprey larvae

Joy Polston-Barnes & Jessica Olmstead
Washington State Department of Natural Resources

Research Introduction

Pacific Lamprey were petitioned and denied for listing under the Endangered Species Act in 2003 due to lack of information on the distribution and age structure of the population (USFWS 2004). As a result, multiple agencies in Washington, Oregon, Idaho, and California have partnered to collect distribution data, restore, enhance, and open river habitat for the Pacific Lamprey. The Washington State Department of Natural Resources (WDNR) manages river bedlands throughout Washington state, state-owned aquatics lands. WDNR has been considering what conservation efforts the agency can take to support efforts to increase lamprey abundance in river systems. Physically excavating and electroshocking to release the larvae from the sediment is currently how abundance and distribution estimates are obtained. It is unknown if environmental DNA (eDNA) is a reliable method to determine distribution and abundance of Pacific Lamprey larvae in field collected river sediment samples. This research aims to address data gaps for species recovery efforts by determining if this sampling method is a tool that can be used to determine the probability of larvae presence.



Research Questions

- 1) Does eDNA analysis of riverbed sediment accurately identify presence and abundance of lamprey larvae?
- 2) How does eDNA sediment analysis compare to other methodologies used to identify the presence of lamprey larvae, including eDNA water analysis and electrofishing?

Study Area

The Nisqually River was selected for sampling because it has been documented that Pacific Lamprey use the river for all life stages. It was also a good geographic location for partners completing various field and laboratory components of the research.

Sampling occurred on July 30th – August 2nd, 2018, during annual low flow and outside of the expected lamprey and salmonid spawning activity. Eight sites were selected for this study.

Methods

- Each site consisted of three sampling grids within habitat that was previously identified. The shape of each grid was dependent on shoreline characteristics and water presence.
- Sediment samples (n = 12) were taken approximately 0.75 m apart and then mixed together for a composite sample. All samples were taken down river of the next sample (Fig. 1).
- Three replicates were taken from the composite and put into individual sample tubes (50 ml) and stored in an ice-chest for lab submittal (Fig. 2, A).
- Electrofishing for larvae (protocol adapted from USFWS & Yakama Nation) started at the most downstream site and grid, working upstream, making sure to pass over and within 0.75 m of areas flagged from sediment sampling (Fig. 2, B).
- Three people were required, one to e-fish and two to catch larvae. Captured larvae were counted (Fig 2., C) and body lengths estimated based on size class (<80 mm and >80 mm; Fig. 3).
- A water sample was taken at each site, just above all three grids. Water was stored in a cooler separate from sediment samples. A water control was also placed into the cooler. The water will be processed for eDNA.
- All larvae were released immediately following completion of a site. The goal is to quantify larvae biomass at each grid and compare to eDNA cell count findings (T. Liedtke, personal communication, December 6, 2017).

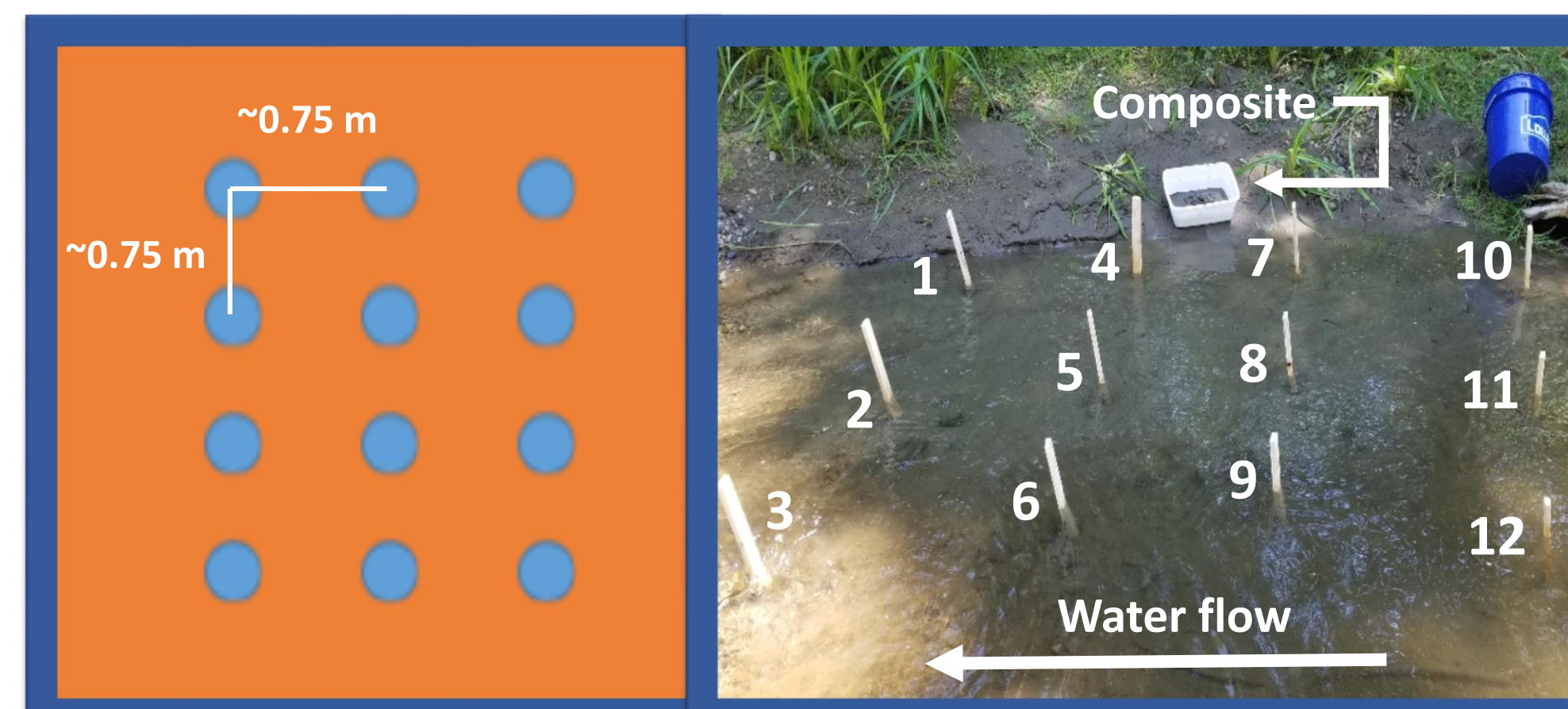


Figure 1. Example of a sample grid and method for sediment collection.

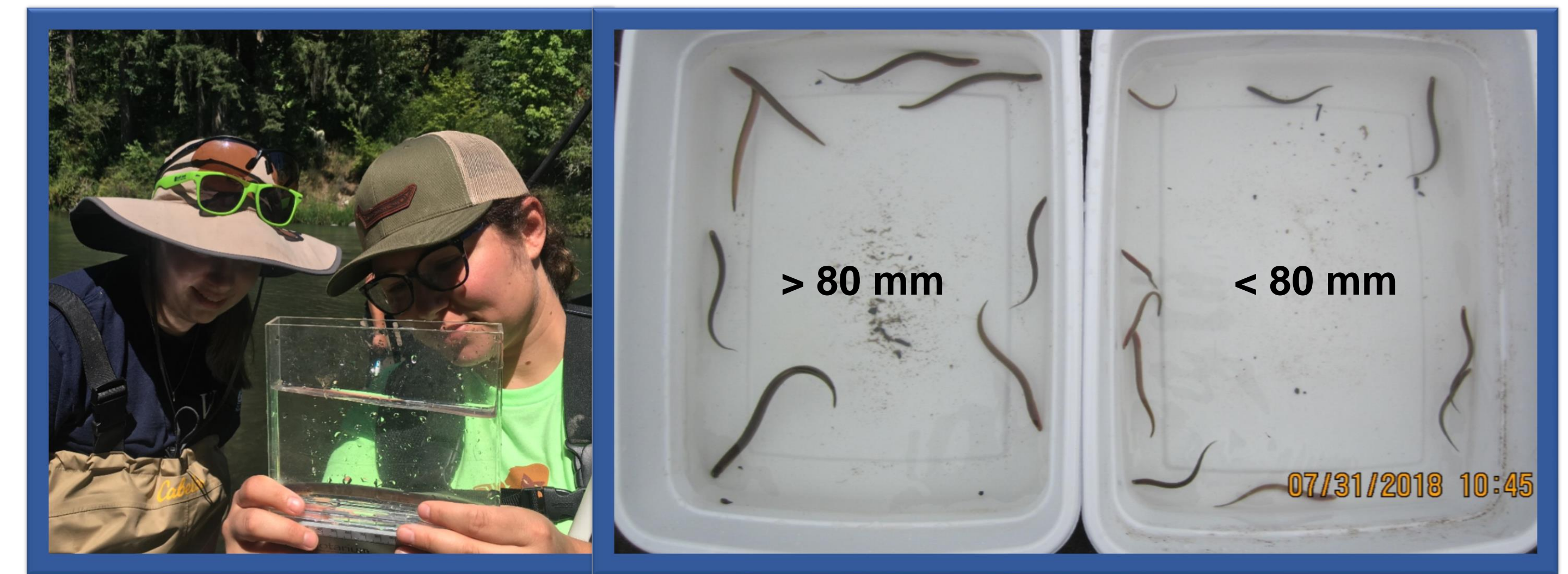


Figure 3. DNR staff using a Photarium to measure body lengths of larvae and separate into size classes.

Habitat Identification

- A data collection mobile app using Survey123 was created for on-site habitat characterization data collection at each sample grid.
- Water quality parameters were measured at every eDNA water sample location using a portable YSI Meter (Pro DSS). This included pH, temperature, DO and conductivity.
- Habitat type (island, side channel, alcove, edge of main channel, or main channel) of each site was qualified and described (Fig. 4).
- In stream or shoreline dynamics such as organic material, woody debris, etc. was recorded (Fig. 5).
- Sediment was collected from the composite after eDNA collection for sediment grain-size analysis to be processed separately by WDNR.



Figure 4. Photo of a site with edge of main channel habitat and shrub grass shoreline vegetation.



Figure 5. Photo of a site with side channel habitat and large woody debris.



Figure 2. DNR staff completing sampling: A) collecting replicates from composite, B) electrofishing, C) counting captured larvae.

eDNA in Sediment

The Washington State Department of Fish and Wildlife (WDFW) Molecular Genetics Laboratory will process 72 sediment samples and 16 water samples. Samples will be tested for the presence of: Pacific Lamprey, and two other native lamprey species found in the river, *Lampetra* spp.; Western Brook Lamprey (*Lampetra richardsonii*) and River Lamprey (*Lampetra ayresii*) cannot be differentiated at this time.

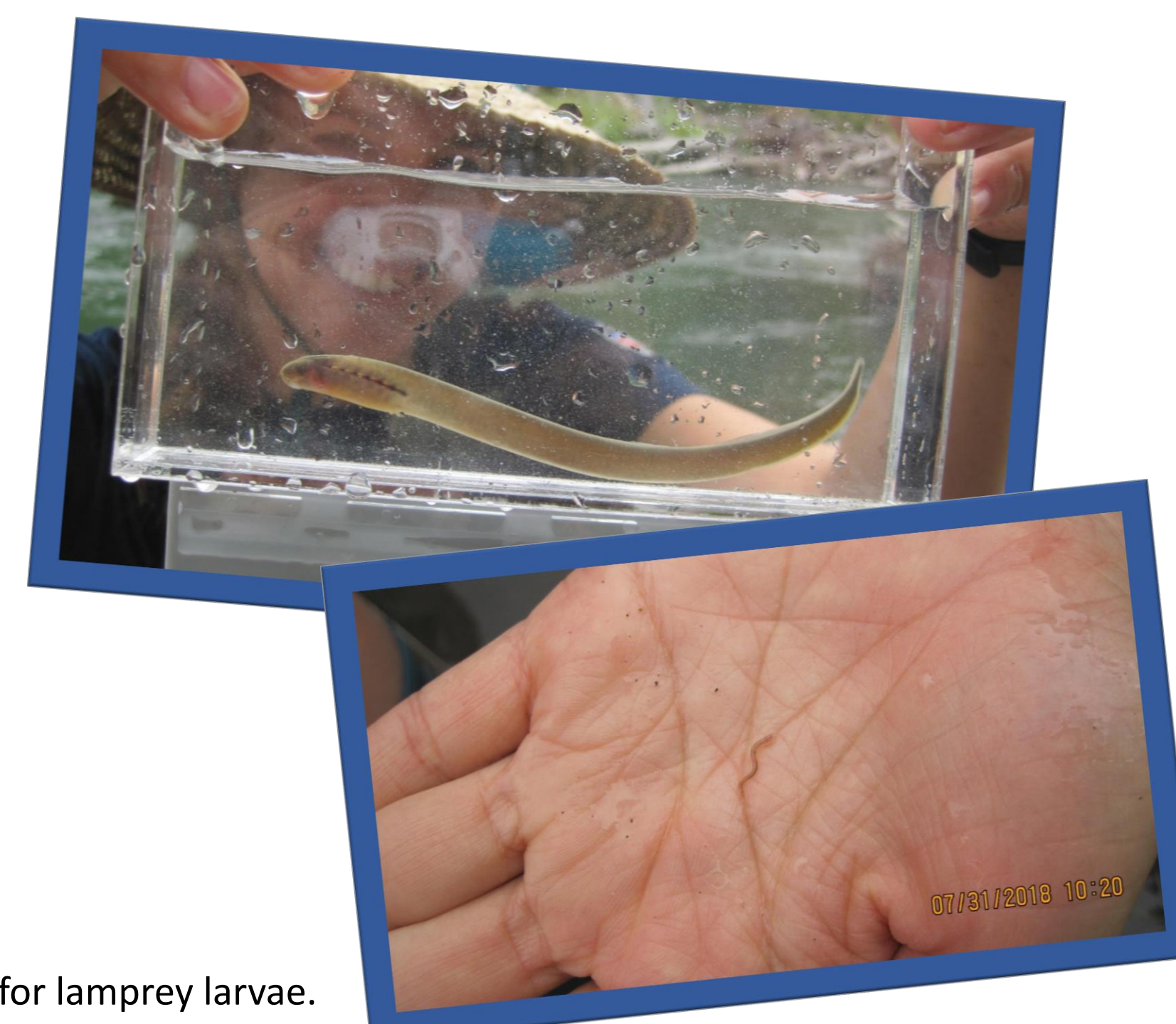


Figure 6. Size variation observed for lamprey larvae.

Results Coming Soon

Results from eDNA analysis are expected to be complete in January 2019. Preliminary lab results show eDNA can be found in the sediment where lamprey larvae are present. Final eDNA and electrofishing results will be assessed and compared at each site and between sites. All data will be put into an occupancy model and results will be reported and analyzed. Further research recommendations will be determined. Conclusions will be made if WDNR can apply the sampling methods on a site by site basis for the management of state-owned aquatic lands.