

## Pilot Study Aquatic Invasive Species Monitoring Program

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**ISSUE:** A critical element for the management of public lands and native aquatic species is the identification and eradication of aquatic invasive species (AIS). USDA Forest Service (USFS) Region 6 (R6) has worked with the PNW Research Station to develop a cost-effective Aquatic Invasive Species Monitoring Program. This program leverages current region-wide field-based efforts that represent wadeable stream reaches across the region. However, additional sampling is needed of non-wadeable areas (i.e. lakes and non-wadeable rivers) with a high risk of aquatic invasive species introduction. The proposed Pilot Monitoring Program will contribute to filling this gap by using eDNA methods in a consistent and rigorous sampling design.

**PROPOSAL for PILOT STUDY:** To visit high risk locations on 2 west-side (Siuslaw and Willamette NF's) Forests to develop eDNA monitoring techniques for 26 USFS R6 AIS Focal Species in non-wadeable freshwater locations (Figure 1). AREMP has agreed to facilitate data collection on these forests.

**GOALS:** 1) evaluate eDNA field techniques for use in high risk locations; 2) develop appropriate sample designs for large water bodies (lakes and non-wadeable rivers); 3) evaluate the probability of detection for different species as a result of water volume processing; 4) develop community-scale eDNA sequences targeting aquatic invasive species.

**BACKGROUND ON eDNA:** Identifying species that are present in water samples from residual DNA, referred to as eDNA, could provide a breakthrough for the monitoring of aquatic species of concern and invasive species, and would help managers plan for multiple

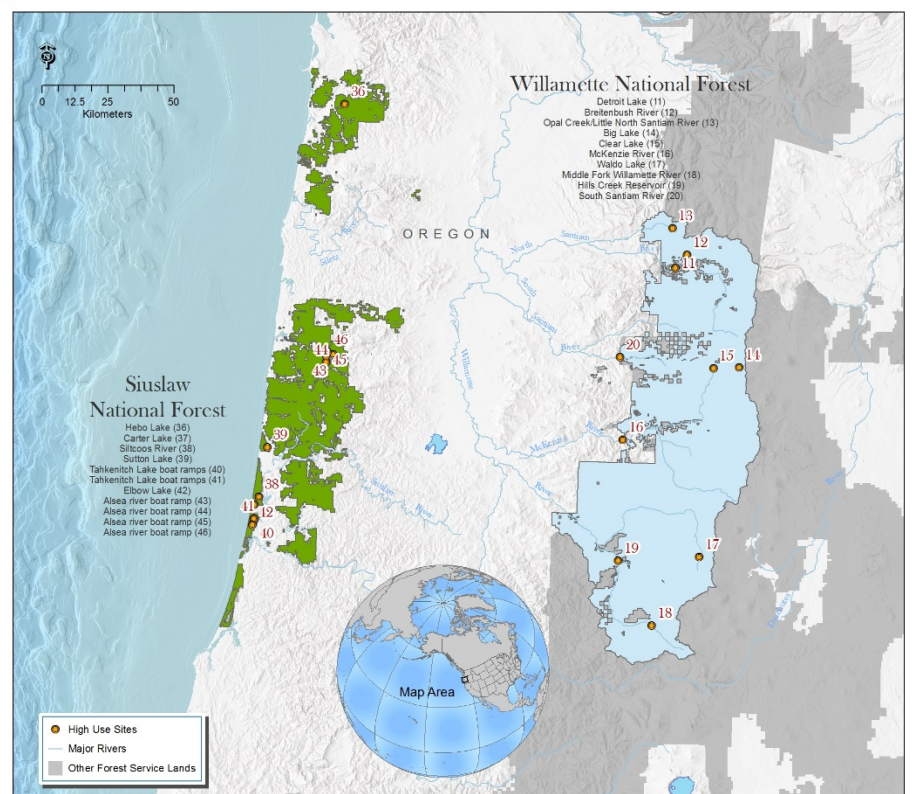


Figure 1 Aquatic Invasive Species Monitoring Program Pilot Study high use sampling locations on the Siuslaw and Willamette National Forests.

management objectives. To identify aquatic invasive species in Region 6 of the USDA Forest Service (Oregon and Washington), using eDNA screening, regionally-specific primers or targets for groups of aquatic taxa of interest must be developed (see Appendix 1 for Focal Invasive Species List for Region 6). This requires vouchered specimens to be collected for each species ultimately adding to a 'genomic' library.

Genomic libraries contain a collection of genomic information. They are used to identify populations or species of interest in a sample using partial or complete sequencing of target DNA regions. Primers are short pieces of DNA that are synthesized to be nearly identical to target genes used in Polymerase Chain Reaction (PCR) assays. They are designed to match DNA segments that are diagnostic to specific target groups, such as unique populations of a species, species from a genus, or even genera and families. Although some species in the Pacific Northwest are represented by some DNA sequence information, most species of interest have had only limited population sampling, and substantial samples of important species many lack genetic information in public databases.

## **FIELD METHODS:**

### Goals 1-3

Survey sites: Surveys will focus on High Use locations identified by the Siuslaw and Willamette National Forest (Figure 1). This includes lakes and non-wadeable river reaches. To leverage existing eDNA surveys occurring at AREMP watersheds, we will select sites where AREMP is already conducting eDNA surveys in the wadeable portions of the watersheds. This will allow us to explore potential questions of detectability in water samples from simultaneous samples in multiple locations within a watershed. Sample sites will be GPS'd in the field, and must occur at public access locations.

Sampling methods: River reaches identified as High Use locations will be surveyed at one site. Lakes will be surveyed at up to 5 locations including: river inflow (up to 2); outflow; boat launches. At each survey site, three samples will be taken to answer the question of how important the volume of processed water is in organism detection. Samples will be taken using 1L, 30L, or 60L as processed with a peristaltic pump. Contamination between sites will be avoided by requiring field crews to decontaminate between sites using a 50% bleach solution and by sampling from piers or docks to avoid across-site contamination.

### Goal 4

Collection of genetic information for invasive aquatic species of interest for a regionally-specific genomic library (Table 1). This work will leverage efforts by partners, in particular, ODFW is currently funding the development of a genomics library for native fishes including salmon/trout, lamprey, suckers, minnows, troutperch, sturgeon, and sculpin. In this project, we would contribute by developing a genomic library for invasive *warmwater fishes* (i.e. bass, catfish, sunfish); as well as native *amphibians*, including aquatic-dependent frogs, salamanders, and newts; and native *invertebrates*, including crayfish and mollusks.

Table 1. Tasks with anticipated costs.

<b>Tasks</b>	<b>Costs</b>
Development of eDNA genomic library for aquatic invasive species	\$35,000/24 species
AREMP field crew to visit west-side sites	AREMP covers costs
FS employees to visit east-side sites	FS covers costs
eDNA sampling gear and supplies	\$15,000
eDNA lab processing (supplies, genotyping, genotyping labor)	\$35,000/196 samples
Data output and analysis	\$30,000
	<b>Total \$115,000</b>

**ANTICIPATED ANALYSIS:**

Research questions that are directly linked to the goals of the study will be evaluated. In particular, we will 1) evaluate eDNA field techniques for use in high risk locations. In lakes, we will compare eDNA results at multiple sites within the lake to quantify detection variability across species. In non-wadeable river segments, we will compare species detection variability with upstream samples taken as part of standard AREMP monitoring practice. The results of these comparisons should begin to answer questions about survey design. 2) To evaluate the probability of species detection as a result of water volume processing (3 samples were collected at each site, each by processing different amounts of water), we will do site-scale, forest-scale, and between forest comparisons of: a) individual species detected, and b) total number of species detected.

**ANTICIPATED OUTCOME:**

This pilot study is intended to inform a regional aquatic invasive species monitoring program. The new technology being used in this monitoring design requires investigation of effectiveness and evaluation of survey design. The pilot project is anticipated to answer some questions about both of these elements that are necessary for a successful invasive species monitoring design.

Appendix 1 – Focal Aquatic Invasive Species List for Region 6 – 2016.

Type	Common name	Genus species	Species Code	Preliminary species barcoding to run eDNA analysis available?
Aquatic animals	New Zealand mudsnails	<i>Potamopyrgus antipodarum</i>	POAN	Yes
	Zebra mussels	<i>Dreissena polymorpha</i>	DRPO	Yes
	Quagga mussels	<i>Dreissena rostriformis bugensis</i>	DRRO	Yes
	Rusty Crayfish	<i>Orconectes rusticus</i>	ORRU	Yes
	Red Swamp Crayfish	<i>Procambarus clarkii</i>	PRCL	Yes
	Ringed Crayfish	<i>Orconectes neglectus</i>	ORNE	Yes
	Bullfrog	<i>Rana catesbeiana</i>	RACO	Yes
	Northern Crayfish	<i>Orconectes virilis</i>	ORVI	Yes
	Nutria	<i>Myocaster coypus</i>	MYCO	Yes
	Asian Clam	<i>Corbicula flumina</i>	COFL	No; only genus information found
	Chinese mystery snail	<i>Cipangopaludina chinensis</i>	CICH	Yes
Big Eared Radix	<i>Radix auricularia</i>	RAAU	Yes	
Aquatic plants	Yellow Flag Iris	<i>Iris pseudacorus</i>	IRPS	Yes
	Hydrilla	<i>Hydrilla verticillata</i>	HYVE	Yes
	Nonnative Milfoils	<i>Myriophyllum species</i>	MYSP	Yes
	Yellow Floating Heart	<i>Nymphoides peltata</i>	NYPE	Yes
	Giant Salvinia	<i>Salvinia molesta</i>	SAMO	Yes
	Giant Reed	<i>Arundo donax</i>	ARDO	Yes
	Brazilian Elodea	<i>Egeria densa</i>	EGDE	Yes
	Didymo	<i>Didymosphenia geminata</i>	DIGE	Yes
	Flowering rush	<i>Butomus umbellatus</i>	BUUM	Yes
	Common reed	<i>Phragmites australis</i>	PHAU	No; only one relevant entry in Genbank
	Curly-leaf pondweed	<i>Potamogeton crispus</i>	POCR	Yes
	Purple Loosestrife	<i>Lythrum salicaria</i>	LYSA	Yes
	Garden Loosestrife	<i>Lysimachia vulgaris</i>	LYVU	Yes
Water primrose	<i>Ludwigia spp.</i>	LU	No; only genus information found	