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The Technical Newsletter of the National Stream and Aquatic Ecology Center

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Aquatic Biodiversity from a Bottle of Water: Using eDNA to Understand Species Richness

Many aquatic species warrant assessment and monitoring by managers, including fish, amphibians, mollusks, crustaceans, insects, and animal and plant pathogens. Logistical challenges and financial costs associated with monitoring so many aquatic species is becoming an increasingly overwhelming task, especially as demand for this information outpaces the available funding and taxonomic expertise required for accurate species-level identification. There is an urgent need for new approaches that provide rapid and accurate assessments of presence and absences for multiple aquatic species across diverse taxa.

Environmental DNA (eDNA; Figure 1) metabarcoding is increasingly used to meet this important need. This method can evaluate DNA diversity in a sample of stream water by identifying residual DNA from cells, organelles, or free DNA that is left

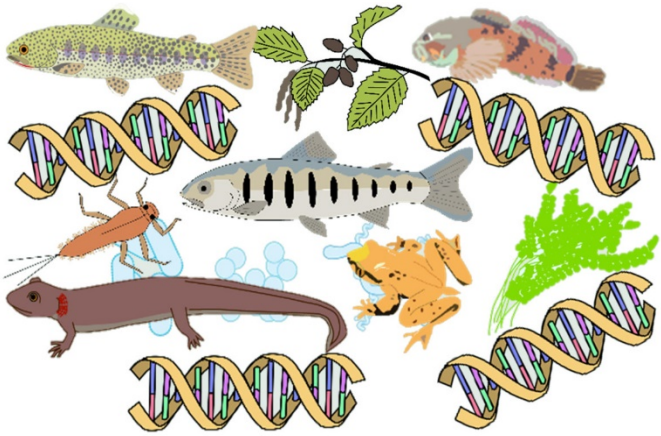

Figure 1 Environmental DNA (eDNA) is DNA that is left behind in the environment by biota. Managers can use eDNA to identify aquatic species and their relative abundance in streams.

StreamNotes is an aquatic and riparian systems publication with the objective of facilitating knowledge transfer from research & development and field-based success stories to on-the-ground application, through technical articles, case studies, and news articles. Stream related topics include hydrology, fluvial geomorphology, aquatic biology, riparian plant ecology, and climate change.

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behind in the water. Generally, eDNA metabarcoding uses the polymerase chain reaction (PCR) to amplify short, taxonomically informative genomic regions (DNA "barcodes") from eDNA. These PCR products are then sequenced to reveal broad taxonomic diversity in groups, such as 'all ray finned fish' or 'all amphibians' (Valentini et al. 2016). While there are shortcomings associated with eDNA metabarcoding as currently practiced, such as false negatives (Jane et al. 2015, Deiner et al. 2017) and extensive bioionformatic analyses (Schwartz et al. 2017), eDNA metabarcoding has been shown to identify a larger number of species than either traditional sampling methods (Thomsen et al. 2012, Valentini et al. 2016, Deiner et al. 2016) or newer DNA-based methods (e.g., qPCR or ddPCR), and at a cost that is comparable to both methods. In addition to these advantages, eDNA sampling methods are non-lethal and impose minimal habitat disruption.

Streams act as 'conveyer belts' that integrate aquatic and terrestrial biodiversity information – streams can be used to estimate species richness across the land-water interface (Deiner et al. 2016). Modern genome sequencers have an enormous capacity to detect DNA sequences – currently, one-half billion DNA sequences per sample – but most eDNA metabarcoding methods amplify only one to two genomic regions that identify focused taxa to the level of genus or higher (Thomsen et al. 2012, Valentini et al. 2016, Deiner et al. 2016). We are exploiting this massive capacity to identify a larger number of targets. By using parallel PCR amplification of 48 different DNA barcodes, we can combine 'taxon-general' (e.g., orders, families, genera) and 'taxon-specific' (e.g., genera, species) screening methods into a single assay that maximizes our ability to identify species of fish, amphibians, mollusks, crustaceans, insects, and animal and plant pathogens (Figure 2). Advantages of this approach include: (1) species identification can be made using multiple independent genetic markers, rather than based on a single molecular (qPCR, ddPCR) assay; (2) common and endangered species from different taxonomic groups (even kingdoms) can be detected in a single analysis; (3) the assay can be scaled to include hundreds of samples per DNA sequencing run; and (4) diverse user groups can coordinate collections to meet multiple management objectives (e.g., anadromous fish management and forest pathogen screening).

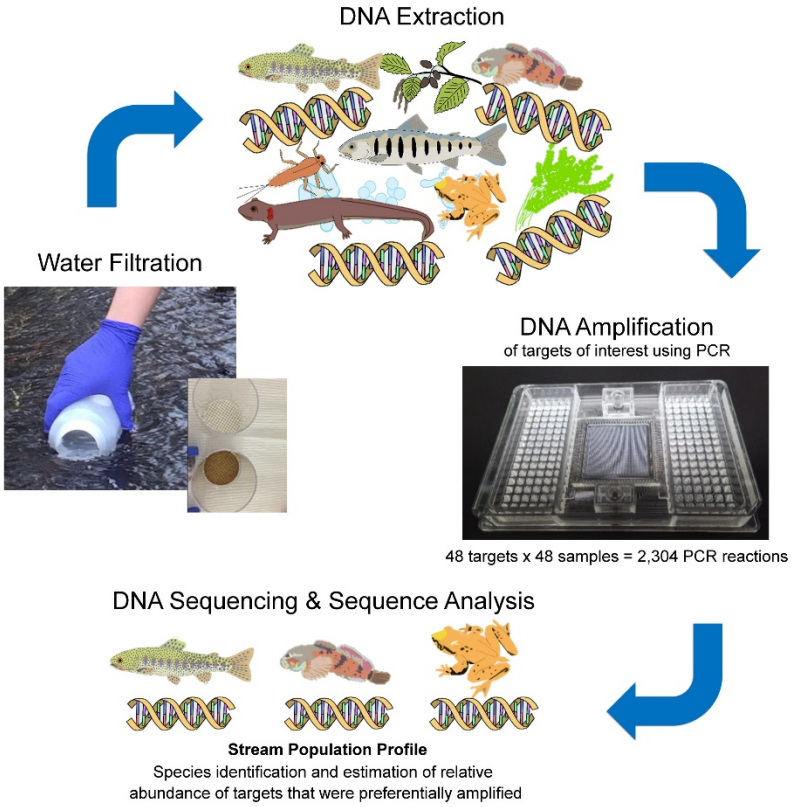

Figure 2: eDNA metabarcoding work-flow diagram used by the Forest Service Pacific Northwest Research Station, including water filtration in the field, DNA extraction in the lab, DNA amplification, and DNA sequencing and analysis, which includes mapping sequences to known species.

At present, we are testing this 'massively parallel' eDNA metabarcoding approach on diverse streams, lakes, and reservoirs across the Pacific Northwest (Figure 3; Figure 4). Preliminary results from our proof-of-concept studies show that comprehensive aquatic biodiversity assessments are possible for fish (sea-run and resident), important forest pathogens, aquatic insects, crayfish, and amphibians. These projects also consider invasive species (plant and animal) and genetic diversity in target fish and amphibians.

Ultimately, results from our work will link science to management by delivering science-based, region-specific information of multiple aquatic species to stakeholders, allowing them to make informed decisions. Currently in development is technology that managers and stakeholders can use for aquatic monitoring projects that would

allow them to understand whether a species is present, to evaluate their relative abundance, and to determine species distributions, especially as species presence, abundance, and distributions change in response to management activity, invasive species, and future climate change.

Management Implications

- Comprehensive aquatic biodiversity assessments offer a breakthrough for the monitoring of aquatic species, including the simultaneous identification of fish, amphibians, mollusks, crustaceans, insects, and animal and plant pathogens.
- Environmental DNA (eDNA) samples provide an integrated signal of presence for many species, and this signal depends on biotic and abiotic conditions of each stream system.
- Our work expands narrower 'single species' assays by using high-throughput sequencing to enable a broader scope of eDNA analysis. This allows us to detect diverse taxa and provide data that informs conservation decisions for a wide range of riparian and aquatic taxonomic groups.

More Information

More information on this work is available at the storymap Leveraging Leftovers: Using Multispecies eDNA to Track Freshwater Species.

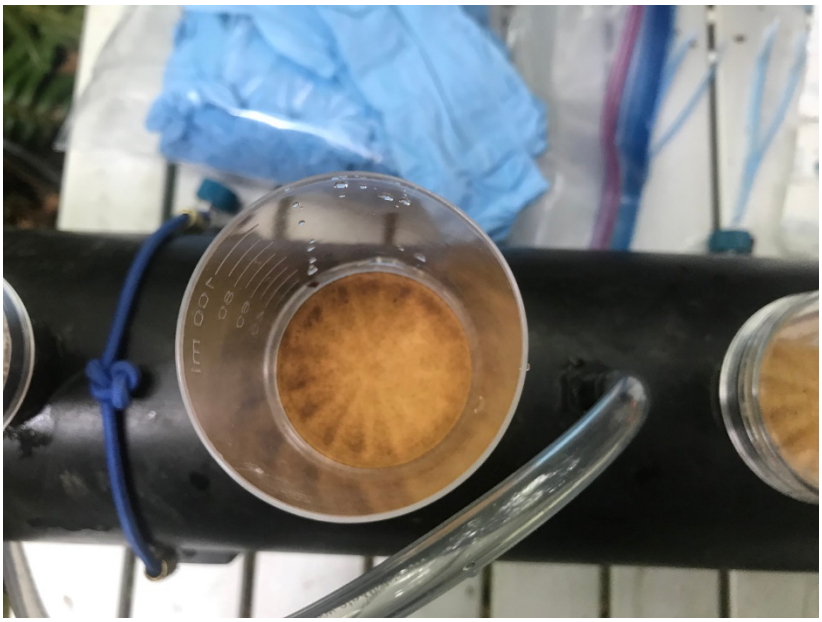

Figure 3: Filtering of eDNA water samples in the field generally requires 5 to 45 minutes, with 3 L of clean stream water for each sample. More time is required when samples have more sediment, algae, or debris.

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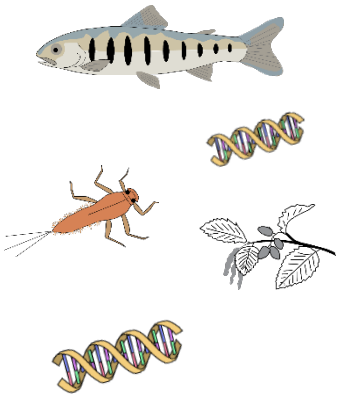

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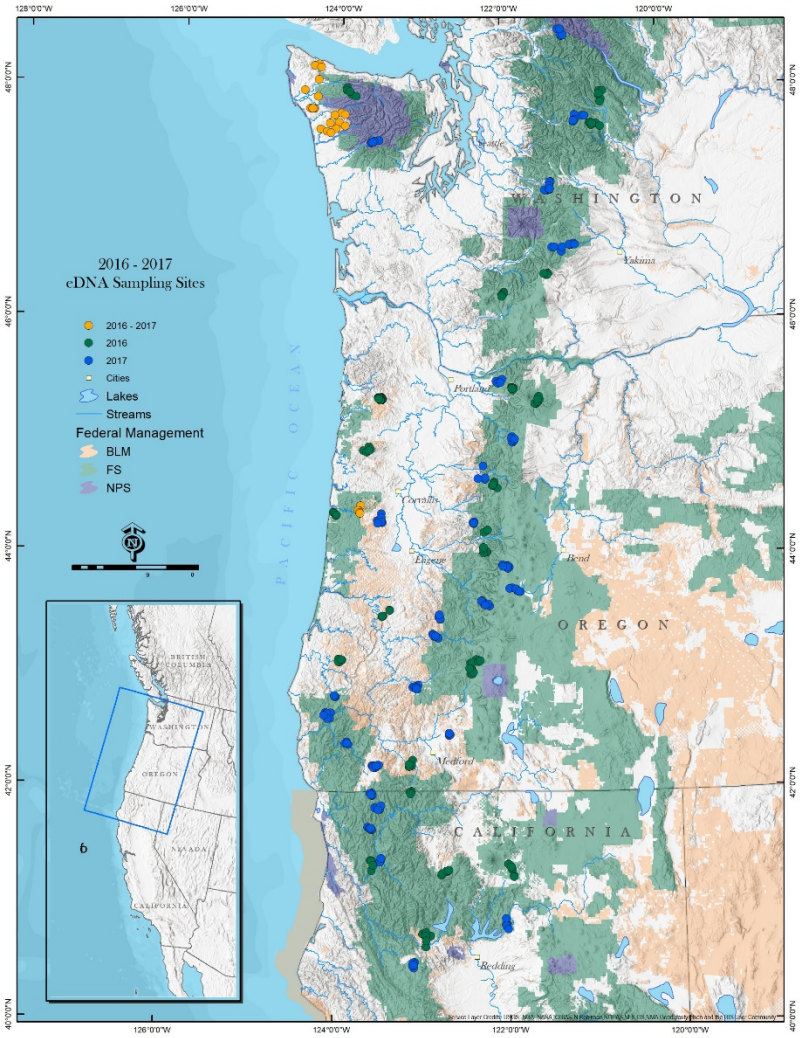
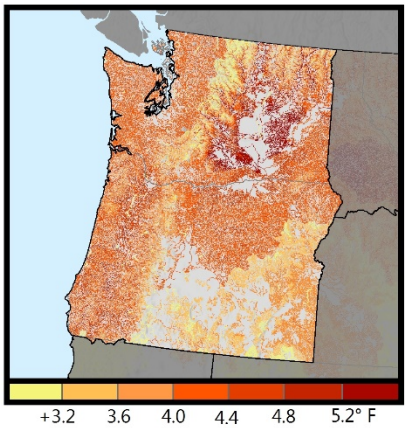


Figure 4: Multispecies eDNA sampling sites across the Pacific Northwest. Samples collected by trained crews through the Forest Service Aquatic and Riparian Effectiveness Monitoring Program, the Olympic Experimental State Forest, and the Pacific Northwest Research Station.

Notices and Technical Tips

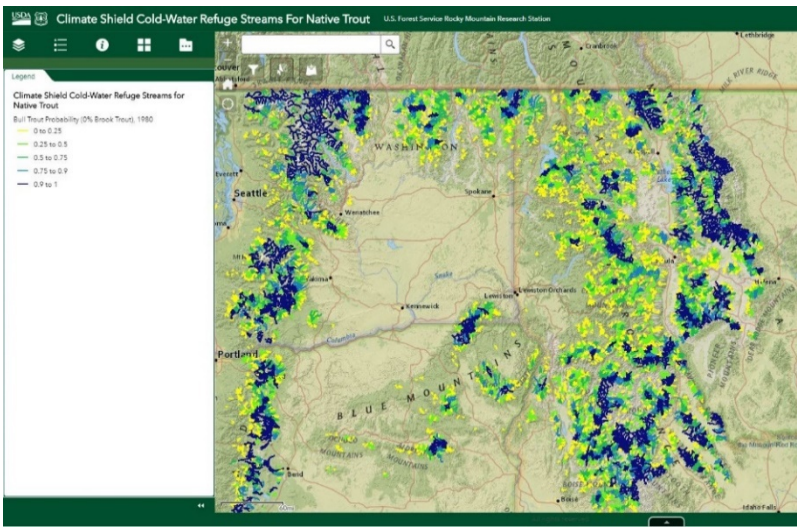
- **Direct technical assistance from applied scientists at the National Stream and Aquatic Ecology Center** is available to help Forest Service field practitioners with managing and restoring streams and riparian corridors. The technical expertise of the Center includes hydrology, fluvial geomorphology, riparian plant ecology, aquatic ecology, climatology, and engineering. If you would like to discuss a specific stream-related resource problem and (if needed) arrange a field visit, please contact a scientist at the Center or David Levinson, the NSAEC program manager.\

- This spring, the Rocky Mountain Research Station (RMRS) and the Office of Sustainability and Climate (OSC) published two new datasets in the Enterprise Data Warehouse and on the Forest Service's Geospatial Data Discovery page:



  - NorWeST modeled stream temperatures for 1.7 million stream segments spanning the western United States, which includes scenarios for historical and future climate periods. The dataset underpinning the scenarios was aggregated from contributions by hundreds of biologists and hydrologists employed by more than a hundred resource agencies, and includes more than 20,000 stream locations with temperature measurements, and hundreds of millions of hourly records. This work was previously published in Water Resources Research, with the full datasets available through the RMRS website. Now, this information is more readily searchable and accessible through the Forest Service's authoritative data portals. These data can also be queried, filtered, and downloaded through a custom ArcGIS Online application.

  - Climate Shield species distribution models predicting long-term refugia for bull trout and cutthroat trout populations in the western United States. These models use the NorWeST scenarios with state and federal biological species occurrence datasets to help managers and scientists evaluate where native trout populations are most likely to persist under future stream conditions. These models include both projected changes in temperatures, and scenarios for the expansion of nonnative brook trout populations. The dataset and supporting materials are available on the RMRS website, as is the associated peer-reviewed paper that was published in the journal Global Change Biology. Like the NorWeST data, the Climate Shield modeled scenarios are now available through the Enterprise Data Warehouse and the Geospatial Data Discovery page. Additionally, an ArcGIS Online application makes it possible for users to query, filter, and download these datasets. By working to improve the visibility and usability of these two datasets, we can help scientists and managers to plan for future changes in stream conditions in the western United States.



Pilot Study for Multiple Aquatic Invasive Species Monitoring

Public lands in the western U.S. are managed for ecological sustainability and the well-being of local communities. An important part of this mandate for range and forest managers in the Pacific Northwest is the conservation, restoration, and recovery of aquatic habitats that support native fishes, amphibians, mussels, and macroinvertebrates. One element of this work is vigilance in the early detection and eradication of aquatic invasive species that have the potential to compromise local habitats, native species, and aquatic and riparian ecosystems. Aquatic invasive species are known to have devastating ecological (Vitousek et al. 1997) and economic impacts (Pimentel et al. 2000) if they are allowed to develop strongholds outside of their native range. In freshwater settings, one primary agent that distributes aquatic invasive species are the boots and boats of recreationalists (Johnson et al. 2001). This means that some of the highest-risk locations for potential invasion by aquatic invasive species are the places most frequented by fishers, boaters, and campers. This includes many locations on national forests and grasslands (Figure 5) and Bureau of Land Management (BLM) lands. To promote management of moving waters under federal jurisdiction, the Pacific Northwest Region (6) of the USDA Forest Service has developed an Aquatic Invasive Species Monitoring Program, of which this pilot project is a part. With a comprehensive monitoring program, local communities and mangers will have information on early detections of aquatic invasive species on national forests and BLM lands. Ultimately, this information will help to sustain productive aquatic communities and habitats for culturally and economically important fishes such as Pacific salmon, trout, and lamprey.

Forest Service Region 6 encompasses 17 national forests (and a national scenic area) distributed across Oregon and Washington. These national forests provide recreational opportunities, are critical habitat for a variety of threatened and endangered species, and serve as the major source of municipal and agricultural water for the Pacific Northwest (USDA Forest Service 1990). Region 6 has worked with fish biologists and geneticists at the Pacific Northwest Research Station to develop a cost-effective Aquatic Invasive Species Monitoring Program. This program leverages current region-wide field-based efforts that survey wadeable stream reaches, the Aquatic and Riparian Effectiveness Monitoring Program (AREMP; Miller et al. 2017) and the PACFISH/INFISH Biological Opinion Effectiveness Monitoring Program (PIBO; Kershner et al. 2004). However, additional sampling is needed for non-wadeable areas (lakes, reservoirs, and non-wadeable rivers) that are at high risk of aquatic invasive species introduction. Identifying the presence of invasives from residual DNA in water samples, referred to as eDNA, could provide a breakthrough for the monitoring of aquatic species of concern and invasive species, and would help managers meet multiple objectives. For more discussion of eDNA, see Penaluna et al. (this issue).

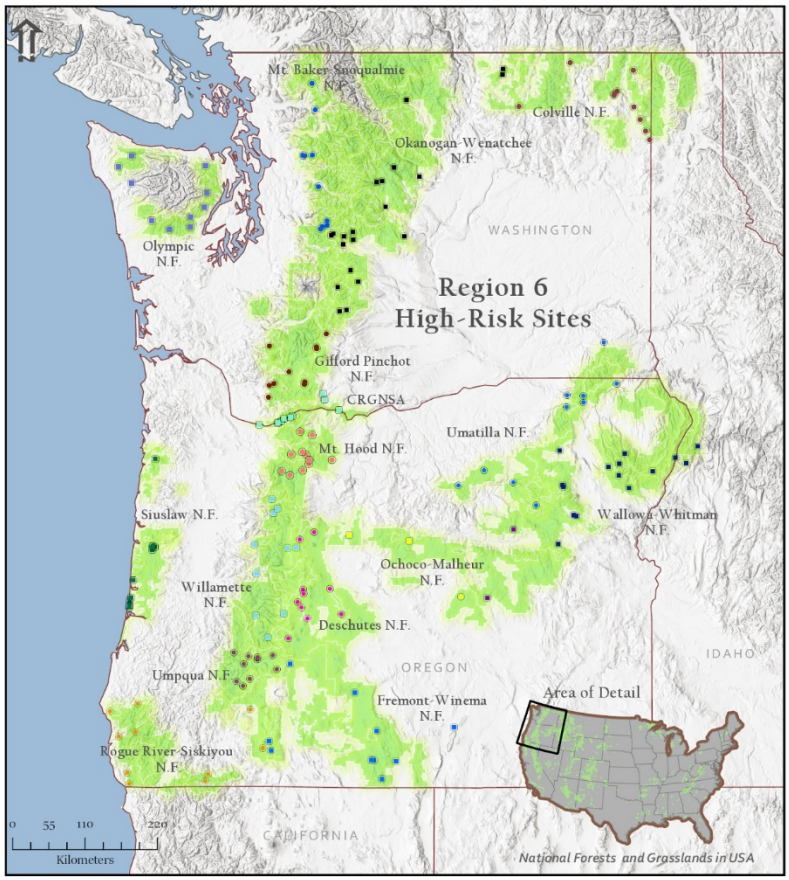


Figure 5: Locations on USDA National Forests in Region 6 identified by forest biologists as being at high risk of invasion by aquatic invasive species due to high recreational use or connection to existing populations of invasives.

The Pilot Monitoring Project is an interdisciplinary and multi-agency effort intended to provide foundational results that will directly inform the design, implementation, and interpretation of a comprehensive Aquatic Invasive Species Monitoring Program. In order to effectively implement such a program using state of the art multi-species eDNA techniques, questions regarding study design and species detectability must be explored. In 2017, the first year of this pilot project, we initiated field studies to demonstrate that eDNA could be sampled in big-water environments (lakes, reservoirs, and non-wadeable rivers; Figure 6). Sample analysis is ongoing and we anticipate that it will reveal the number of replicate samples and quantity of water that needs to be processed when sampling. In addition to field sampling in 2017, we developed primers in the lab for identification of the animals and most of the plants on the Region 6 Aquatic Invasive Species Focal List (Table 1). Although primers may need some initial refinement, once developed they provide the foundation for broad-scale multi-species aquatic invasive species monitoring. In 2018 we are analyzing samples from 2017 and performing additional field surveys that build on what was learned in summer 2017. Currently, field sampling occurs bi-monthly from May to September to test how detection changes throughout the summer months. This is a critical step in designing a region-wide monitoring program so that the sampling can target the best time of the year in different big-water environments across geographic locations.

Figure 6: Lake and large-river environments that are high-risk locations for invasion by aquatic invasive species due to uses such as boating and fishing.

Pilot Project Goals

- Create primers for regional aquatic invasive species
- Evaluate eDNA field techniques for use in high-risk locations
- Develop appropriate sample designs for big-water environments
- Assess the variability in detection for different species

Sample Sites and Field Methods

We are sampling six water bodies during the summer of 2018 (Figure 7), on three national forests (Crane Prairie Reservoir on the Deschutes NF; Lake of the Woods on the Fremont-Winema NF; Tahkenitch Lake on the Siuslaw NF) and on BLM-managed lands (New River, Cow Creek, and the Rogue River). These locations were previously identified by local managers as either high-use locations (making them vulnerable to aquatic invasive species introductions) or as having known populations of invasives (an important criterion to allow for species-detection testing). Chosen sites were on federal public lands and were intended to represent the types of big-water environments (lakes, non-wadeable rivers, and reservoirs) currently outside the sampling frame of existing aquatic monitoring programs. The two lake sites are Tahkenitch Lake (2118 acres) on the Oregon Coast, and Lake of the Woods (1146 acres) near the crest of the Southern Oregon Cascades (Figure 8). Tahkenitch Lake is known to have populations of Brazilian Elodea (*Egeria densa)*, Asian Clam (*Corbicula flumina*), and Yellow

Table 1: 2017 Focal Aquatic Invasive Species List and primer development status for US Forest Service Region 6.

Type	Common name	Genus species	Species Code	Primers Developed
Aquatic animals	New Zealand Mudsnail	*Potamopyrgus antipodarum*	POAN	Yes
	Zebra Mussel	*Dreissena polymorpha*	DRPO	Yes
	Quagga Mussel	*Dreissena rostriformis bugensis*	DRRO	Yes
	Rusty Crayfish	*Orconectes rusticus*	ORRU	Yes
	Red Swamp Crayfish	*Procambarus clarkii*	PRCL	Yes
	Ringed Crayfish	*Orconectes neglectus*	ORNE	Yes
	Bullfrog	*Lithobates catebeianus*	RACO	Yes
	Northern Crayfish	*Orconectes virilis*	ORVI	Yes
	Nutria	*Myocaster coypus*	MYCO	Yes
	Asian Clam	*Corbicula flumina*	COFL	Yes
	Chinese Mystery Snail	*Cipangopaludina chinensis*	CICH	Yes
	Big-eared Radix	*Radix auricularia*	RAAU	Yes
Aquatic plants	Yellow Flag Iris	*Iris pseudacorus*	IRPS	in process
	Hydrilla	*Hydrilla verticillata*	*HYVE*	in process
	Non-native milfoils	*Myriophyllum* species	MYSP	in process
	Yellow Floating Heart	*Nymphoides peltata*	NYPE	in process
	Giant Salvinia	*Salvinia molesta*	SAMO	in process
	Giant Reed	*Arundo donax*	ARDO	in process
	Brazilian Elodea	*Egeria densa*	EGDE	in process
	Didymo	*Didymosphenia geminata*	DIGE	in process
	Flowering Rush	*Butomus umbellatus*	BUUM	in process
	Common Reed	*Phragmites austalis*	PHAU	in process
	Curly-leaf Pondweed	*Potamogeton crispus*	POCR	in process
	Purple Loosestrife	*Lythrum salicaria*	LYSA	in process
	Garden Loosestrife	*Lysimachia vulgaris*	LYVU	in process
	Water Primrose	*Ludwigia* species	LU	in process

Flag Iris (*Iris pseudacorus)*. Lake of the Woods has populations of Yellow Flag Iris. New River is a river/lake system on the southern Oregon Coast (Figure 8) selected for its large population of New Zealand Mudsnail (*Potamopyrgus antipodarum)*. The two river sites are the Rogue River, with populations of Chinese Mystery Snail (*Cipangopaludina chinensis)*, Curly-leaf Pondweed (*Potamogeton crispus)*, Purple Loosestrife (*Lythrum salicaria)*, Ringed Crayfish (*Orconectes neglectus)*, and Yellow Flag Iris; and Cow Creek, on the South Umpqua River, which has populations of Purple Loosestrife and Ringed Crayfish (Figure 8). Crane Prairie (3420 acres) in central Oregon is the only

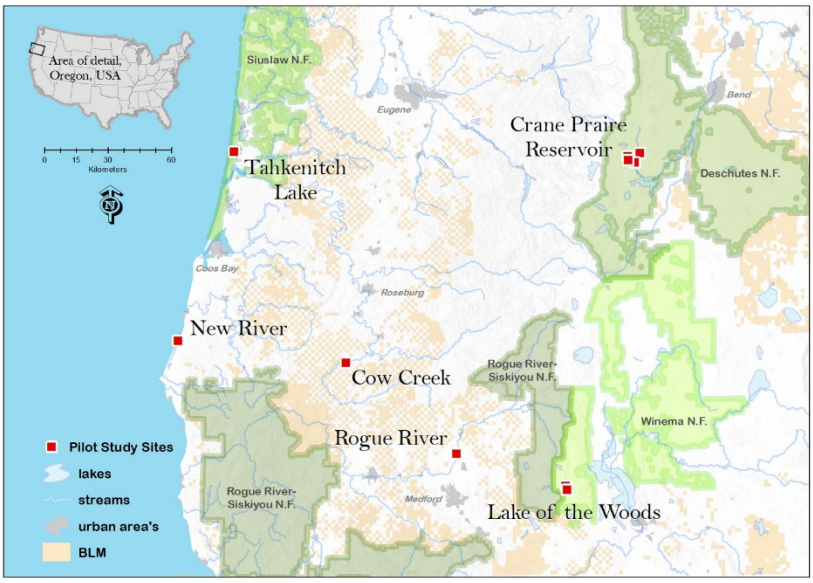


Figure 7: Six sample locations for 2018 summer field work on Pilot Study for Multi-Species AIS Monitoring, where we are collecting bi-monthly eDNA samples to test for detectability throughout the summer season.

reservoir site (Figure 8), and has populations of Eurasian Water-milfoil (*Myriophyllum sp.)*, Big-Eared Radix (*Radix auricularia)*, and Chinese Mystery Snail.

River reaches are surveyed at one boat-launch site. Lakes are surveyed at up to 5 locations, including multiple boat launches distributed around the lake. At each survey site, two types of samples are taken to develop understanding of how important the volume of processed water is to organism detection (Figure 9). Samples are being taken using 500-ml Whirl-Pak®, a 0.45-µm disposable filter, and a Mityvac hand pump. One 30-L sample is taken using an Envirochek®, a 1-µm filter, and a peristaltic pump. Decontamination of equipment between sites requires gloves, multiple rinses for within-site cross contamination, Virkon for site-to-site contamination, and sampling from piers or docks whenever possible to avoid across-site contamination (decontamination process informed by Appendix D of the Guide to Preventing Aquatic Invasive Species Transport by Wildland Fire Operations).

Figure 8: Sample sites from the 6 locations selected for sampling in summer 2018 as part of the Pilot Study for Multi-Species AIS Monitoring.

Anticipated Analyses and Results

The analysis will evaluate eDNA field techniques for use in big-water environments. In lakes, comparison of eDNA results at multiple sites will be used to quantify detection variability across species. An assessment of the probability of detection as a result of water volume processed throughout the summer

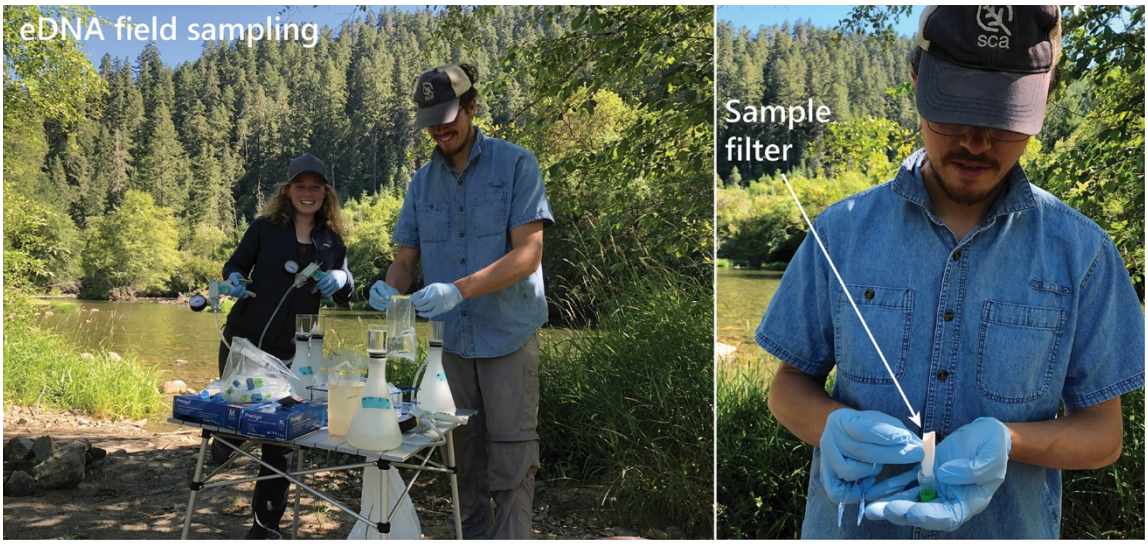


Figure 9: Halie Hajek and James Stockdale complete multi-species eDNA filtering at Cow Creek (BLM) in summer 2018.

season will also be performed, as well as site-scale, forest-scale, and between-forest comparisons of individual species detected and total number of species detected.

The results of this Pilot project will not only help to design more effective aquatic invasive species monitoring, but provide information necessary for implementation of multi-species monitoring by other federal organizations, including the BLM and the Army Corps of Engineers. Further, this work connects developing microbiology and genetic tools to an applied framework that will monitor changes in assemblages of fishes and other aquatic biota. Such information will help managers respond to changes in climate and associated hydrologic regimes as they plan harvest strategies or recreational uses.

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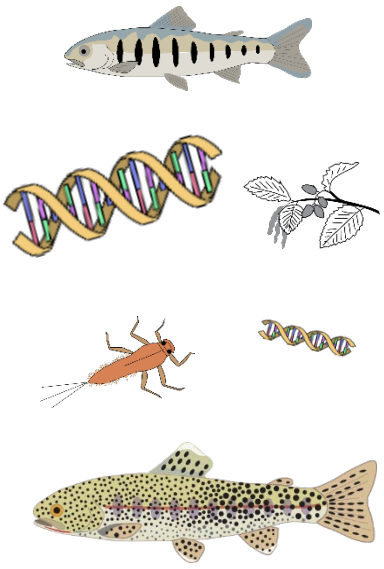
Management Implications

- Monitoring programs may provide forests with an early-warning system for aquatic invasive species detection. However, it is challenging to identify aquatic invasive species when they are new colonists, rather than established populations.
- Environmental DNA collected from water samples for multiple species at one time may provide a useful tool for early detection of aquatic invasive species. However, much still needs to be explored to understand the effectiveness of this technique.
- Multi-species aquatic invasive species monitoring using eDNA may provide a spatially explicit sample of aquatic and riparian habitat, allowing federal land managers to target, identify, and potentially remove invasive species before they become well established, and help them plan and prioritize aquatic restoration projects.



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