

Rocky Mountain Research Station Science You Can Use Bulletin



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Black and White and Shed All Over: How eDNA Analysis Can Help to Answer Your Species Questions

Keeping tabs on the whereabouts of invasive or endangered species in the landscape is an important job for managers. Traditionally, species monitoring has often relied on physical observations of organisms in the field. Often, this requires expertise in species identification. Also, surveys can be labor-intensive, particularly when surveying in remote areas or for organisms that are rare or difficult to find. But today,

there is a powerful new approach that gets around some of these problems: environmental DNA (eDNA).

Organisms continually shed cells containing their DNA into their surroundings. The DNA persists in the environment, where it can be collected as an air, soil, or water sample, extracted, and analyzed for any species of interest. This use of this material for



Environmental DNA sampling has been a major scientific breakthrough for species monitoring. The National Genomics Center for Wildlife and Fish Conservation is a leader in developing eDNA sampling tools and works closely with land managers to apply these tools effectively on the ground (photo: K. Zarn, USDA Forest Service).

SUMMARY

Environmental DNA (eDNA) sampling can infer whether a species is present without the need to physically observe that species. DNA in cells sloughed off from organisms persists in the environment, where it can be collected as a water sample, extracted, and analyzed for any species of interest. Aspects of sample collection may vary depending on the type of water body (pond, lake, river, stream) and area sampled (surface water, very deep lake water, mucky pond water). Study design (where and when samples are collected) can also vary based on research questions. Depending on a biologist's particular question, different types of sample analyses may be chosen to detect the most accurate results. A "targeted" analysis is designed for detection of a particular species with very high accuracy. In contrast, "nontargeted" approaches may be used to broadly characterize community composition. Using targeted approaches, the National Genomics Center for Wildlife and Fish Conservation (NGC), has worked extensively with managers to answer important questions about the presence or absence of particular species. These eDNA projects include: sampling for endangered bull trout to assess the need for endangered species consultations with the U.S. Fish and Wildlife Service (USFWS) in treatment areas; confirming brook trout (an invasive species) eradication in particular stream reaches; and tracking the spread of another invasive species, northern pike, in the Columbia River. The NGC, while continuing important work on fishes, has also expanded the applications of eDNA sampling to survey for rare or recovering mammals, water birds, and amphibians.

species monitoring has been a major technological breakthrough within the last two decades. eDNA ties traditional field-based ecology to exacting molecular methods and advanced computational tools, providing important information about species presence or absence without using traditional, resource-heavy sampling techniques.

The technology has been used primarily to survey for fishes and amphibians. However, samples from water, air, and soil all contain DNA from many species, and applications are expanding to other aquatic taxa, such as mollusks and insects, as well as semi-aquatic and terrestrial mammals like river otter, beaver, wolverine, and lynx.

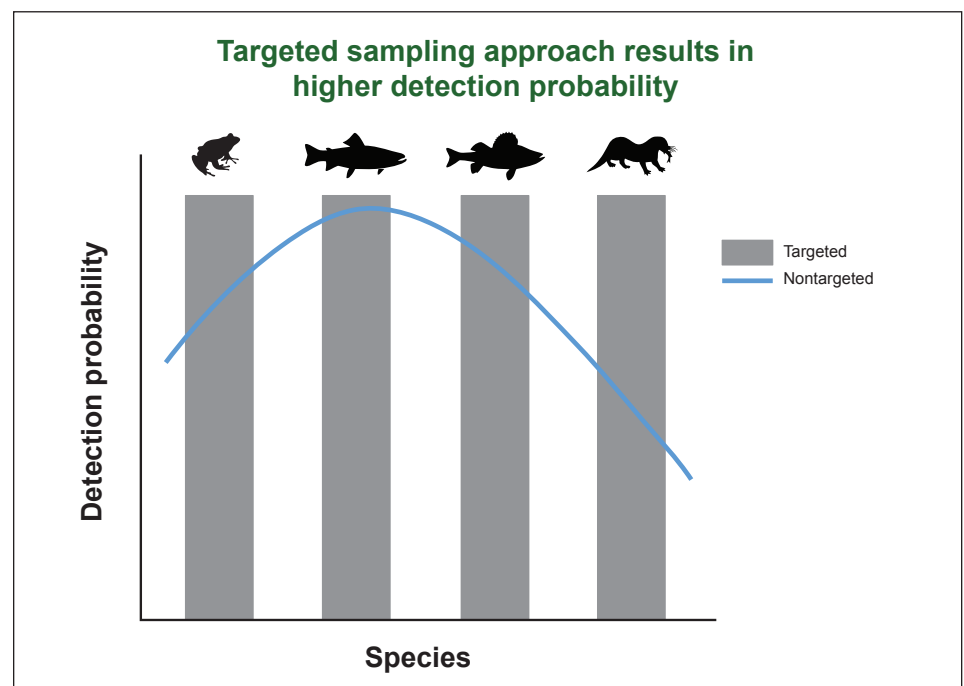
Managers with the USDA Forest Service (USFS) and other organizations are using eDNA to answer important questions, often in collaboration with scientists, at the USFS NGC, which is housed within the Rocky Mountain Research Station (RMRS). According to Mike Schwartz, the RMRS Wildlife Science Program Manager and Director of NGC, “Analysis of eDNA is only one of three major focus areas at the Genomics Center, but its use has been booming over the past few years. It’s one of the most cost-effective and defensible sampling approaches for detecting endangered species and invasive aquatic species, and more and more people are approaching us who are interested in using the technique.”

Even though the word is getting out about the usefulness of eDNA, Schwartz points out that not everybody may be aware of the technique or its potential. “We need to do a better job explaining the basics of the eDNA approach and help our partners realize that not all eDNA techniques are created equal.”

From Targeted to Widespread: Types of eDNA Analysis

Sample collection for eDNA analysis in the field is similar whether you’re hunting for native trout, screening for unknown invaders, or mapping species diversity of amphibians: You collect an environmental sample by scooping soil or snow or filtering air or water. However, what happens in the lab afterwards should be

Sample collection for eDNA analysis in the field is similar whether you’re hunting for native trout, screening for unknown invaders, or mapping species diversity of amphibians: You collect an environmental sample by scooping soil or snow or filtering air or water. However, what happens in the lab afterwards should be matched to the specific question to obtain the most accurate results.



If you are looking for a specific species in your eDNA sample, a targeted approach is preferable because of the higher probability that the species will be detected. A nontargeted approach can be used to answer questions about biodiversity (graphic: T. Wilcox).

Some Ins and Outs of eDNA Sampling in Streams

How much sampling is needed?

Kellie Carim and her colleagues have been working on invasive brook trout eradication in small, headwater streams. There have been several studies where fish are held in cages and researchers measure how far downstream they can detect those fish. “We’ve found that detection of even one or two fish is accurate at about 200–300 meters downstream. If there are areas where managers have concerns, we can add a few more samples to increase our probability of detection.”

Context is important

Every system is different, and it is important that managers consider everything in context. “If you’re looking at a really big river system, the sampling is going to have to change substantially,” explains Carim. “We’ve not applied eDNA in a really large river system to find or detect a single individual. Sitting in the middle of the Columbia River, your ability to capture that DNA is much more difficult than if you’re in a smaller system because of the dilution factor.”

Positive eDNA detections in the absence of fish can happen

Sometimes managers have a positive eDNA detection but can never find the individual fish that may be causing it. According to Carim, “Perhaps there are some fish around that you simply can’t capture. Alternatively, the eDNA being detected may not necessarily be a live fish.” A positive detection can also come from DNA that’s bound to sediment in the system and released into the water again, or from bits of a carcass or the feces of a predator that has recently eaten the species of interest.

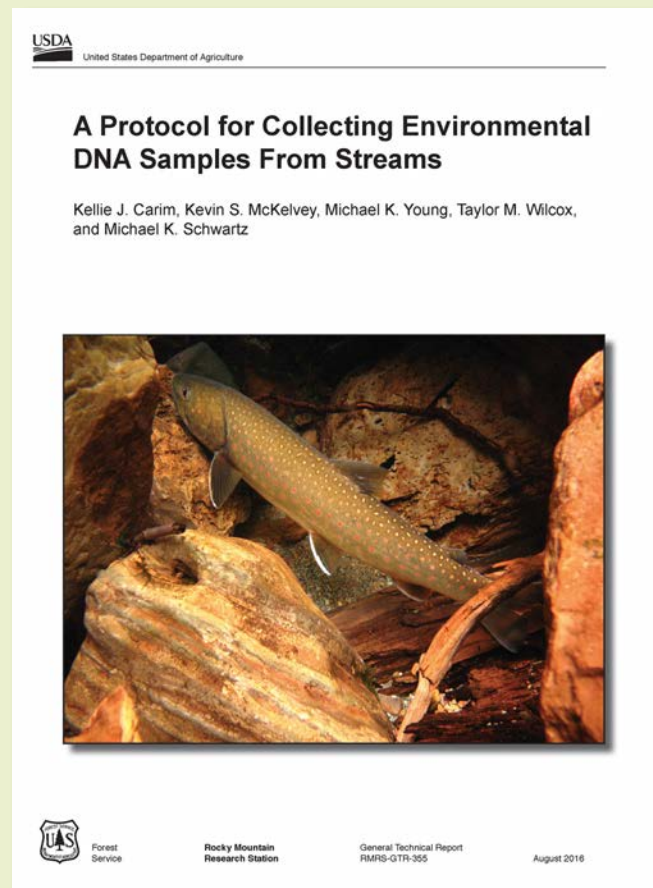
“Our recommendations under those settings are to look at how consistent those detections are,” explains Carim. “A live fish is continually producing DNA and you should get regular, consistent, positive detections. In the other cases, we might get a positive detection, but it would not be consistent over repeated samplings if there were no live fish.”

She recommends that if you get a surprising detection, you should work with your eDNA expert to determine an appropriate

resampling plan. Resampling could combine eDNA with other sampling techniques to pinpoint fish and try to remove or kill them without repeating a chemical treatment.

For more details on how to collect eDNA samples in streams ...

RMRS scientists have developed a concise [how-to guide](#) for sample collection with tips to for avoiding contamination and trouble shooting.



matched to the specific question to obtain the most accurate results.

The lab tools can generally be broken into two main types: “targeted” approaches designed for detection of a particular species and “nontargeted” approaches that detect a group of species

(e.g., “fishes,” “amphibians,” or even a group such as broad as “vertebrates”).

“If you want to pick up DNA from a species that’s rare, then a targeted approach is always going to be your best option because the targeted analysis is optimized for

your focal species,” explains Taylor Wilcox at NGC. “If your question is about community diversity or if there may be members of this community that you might not know to look for, then you would use a nontargeted approach which can reveal unknown community members.” The main shortcoming

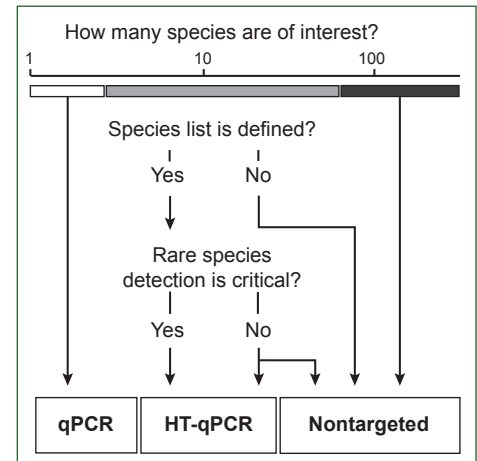
of nontargeted approaches is that they are not as sensitive for detection of each individual species.

The scientists at NGC commonly use quantitative PCR (qPCR) for targeted analyses to answer questions, such as, “Was our brook trout eradication effort in this stream successful?” or “Are threatened bull trout present in this river?” If a manager wanted to use eDNA sampling to ask community-scale questions, such as, “Is amphibian diversity in restored wetlands the same as undisturbed wetlands?” or “Are there novel invasive fishes in this reservoir?” then NGC might suggest a nontargeted approach in the lab. These nontargeted tools include “metabarcoding” and “capture enrichment,” which provide

information about the presence of dozens to hundreds of taxa but do so with less sensitivity for rare species.

NGC has been pioneering a third eDNA option: high throughput qPCR, an approach that is somewhere in between targeted and nontargeted. It starts with a list of species of interest, and a targeted approach is used for each one, but these analyses are done simultaneously, rather than one at a time. A novel application of this technique is using the DNA left by an animal’s foot in a snow track to positively identify which species it came from.

“Because you’re trying to differentiate between closely related species such as bobcat



For one to three species, qPCR is always your lowest cost and most sensitive option. For large numbers of species (> 100) or places where biodiversity discovery is of interest, nontargeted metabarcoding or capture enrichment approaches are most appropriate. At intermediate numbers of species, HT-qPCR may be a useful tool, particularly when rare species detection is critical (graphic: T. Wilcox).

and lynx, it can be difficult to do reliably just based on the features of the track,” Wilcox explains. “If you have a track from a mid-sized carnivore, there’s a suite of different taxa that it could be, but it’s a finite list. So that’s a place where we might use an intermediate approach, because we have a defined list of species and we want to have high sensitivity for detection of each.”

Can eDNA be used to ask questions about the abundance of a species? In general, eDNA sampling is used solely to establish presence or absence of a species. However, the targeted approaches can give you some information about the amount of DNA in a sample. “In some systems, that might tell you something about the abundance of the actual species you’re looking



Video Spotlight



Rocky Mountain Research Station researchers and collaborators have found that they can detect eDNA from snow-track surveys, snow collected at camera stations, and hair samples to detect the presence of Canada lynx, fisher, and wolverine. This has the potential to revolutionize winter surveys for rare species in terrestrial settings by reducing or eliminating misidentifications and missed detections. Video: **Tracking Snow**: <https://www.nationalgeographic.com/animals/2018/11/environmental-dna-snow-helps-track-lynx-rare-animals/>

for, but only if the relationship between the DNA quantity in the sample and the abundance of the species in the field is already really well worked out,” explains Wilcox.

Using eDNA to Detect Species of Conservation Concern: Bull Trout

Environmental DNA analysis can be useful for projects that require endangered species (i.e., Section 7) consultations with the USFWS. The USFS Northern Region (Region 1) has worked extensively with NGC on eDNA-based projects, particularly those involving bull trout.

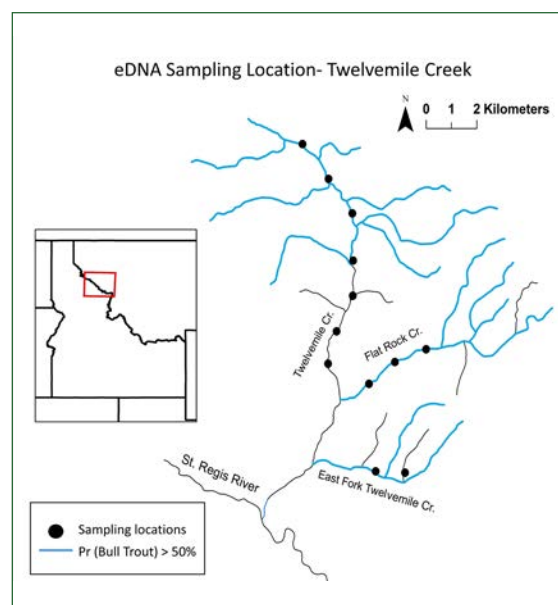
Bull trout is an ESA-listed (threatened) species with a historical range that encompasses many waters across the northwestern United States. Historically abundant, bull trout have declined in many locations from an array of factors like habitat degradation, population isolation, nonnative species invasions, and climate change. Federal listing mandates that agencies have reliable and precise information about the distribution of bull trout in thousands of streams, but bull trout surveys are expensive because the fish are often rare and difficult to collect. Sampling for eDNA in Twelvemile Creek—a tributary to the St. Regis River in the Lolo National Forest in Montana—was among the first of collaborative projects between NGC and USFS Region 1 involving this technology.

One example of eDNA being used for a bull trout Section 7 consultation was a USFS restoration project involving vegetation treatments for fuels and road improvements in an area that included Twelvemile Creek. Bull trout had historically been

identified and documented here in the 1990s but not detected since then despite considerable electrofishing sampling. Still, the USFWS considered this water to be “occupied” spawning and rearing habitat for bull trout. Scott Spaulding, a Region 1 Fisheries



When the Forest Service began a restoration project in habitat of the bull trout, a threatened species under the U.S. Endangered Species Act, managers were able to use eDNA to confirm that the species was not present in the area (photo: R. Hargrave, Oregon Department of Fish & Wildlife).



Environmental DNA (eDNA) can be useful for threatened species monitoring and Section 7 consultations with the U.S. Fish and Wildlife Service. In the Twelvemile Creek Drainage on the Lolo National Forest, dots representing (eDNA) sample locations were chosen based on occupancy models predicting probability of > 50 percent of containing juvenile bull trout (blue reaches). Samples were collected in half a day. All sites sampled were negative for bull trout. Annual monitoring continues during implementation of the Twelvemile–Tamarack (12-Tam) integrated vegetation project to ensure bull trout will not be adversely affected and to be consistent with the ESA consultation (graphic: K. Carim).

KEY FINDINGS

- Environmental DNA sampling has been a major scientific breakthrough for species monitoring. The [National Genomics Center for Fish and Wildlife Conservation](#) has been a leader in developing eDNA sampling tools and in working with managers to apply the tools effectively on the ground.
- Lab analyses of eDNA samples can be targeted for detection of a particular species, or use a nontargeted approach that detects a group of species.
- A targeted approach is the best option when looking for a rare species, due to its greater sensitivity. A nontargeted approach is better for questions related to community diversity, but is not as sensitive for detection of each individual species.
- Environmental DNA sampling has been used primarily for fish and amphibians, but samples from water, air, and soil contain DNA of many species, and applications are expanding to other aquatic taxa, such as mollusks and insects, as well as semi-aquatic and terrestrial mammals including river otter, beaver, wolverine, and lynx.

Biologist and close collaborator with NGC scientists, explains, “When we went to consult with the Fish and Wildlife Service, they wanted us to go through formal consultation for the species, assuming that the species was there and that potential project effects could adversely affect individuals of the species.”

The USFS presented evidence based on recent eDNA sampling that there was a low risk of adverse effects to bull trout presented by this proposed project. “We offered that every year that we’re working on the project, we’ll systematically sample Twelvemile Creek with eDNA using a targeted approach to see if bull trout are in fact occupying that stream. If we confirm ‘occupancy’ using eDNA, there might be some additional design criteria or mitigation measures that the service would want to put in place,” Spaulding explains. “But if they’re not there,

maybe those extra conditions would not be needed.”

The USFWS accepted the proposal. For this project, the use of eDNA has saved the USFS time by not having to go through formal consultation on the species and allowing biologists to put their energy into other projects.

Environmental DNA information has recently been compiled for bull trout: the eDNAtlas. The eDNAtlas supplements other traditional sources of bull trout sampling so that biologists have the best available information on where bull trout reside for project-level planning and ESA consultation. Also, bull trout occupancy models like Climate Shield developed by RMRS (and partly driven by eDNA sampling information) provide managers with broad strategic insight on where they might get the biggest bang for the buck for various types of bull trout conservation efforts, such as road

relocation and riparian restoration, nonnative fish management, or translocation into unoccupied habitat, both now and into the future with predicted climate-forced changes.

Using eDNA to Confirm Invasive Species Eradication: Brook Trout

Native to eastern North America, brook trout were introduced to the western United States in the mid-1800s and spread throughout the area. Only later the negative impacts of these introductions on native western species like cutthroat and bull trout realized. In many areas, managers are now working to remove brook trout and reintroduce native fish. Environmental DNA has proven to be a useful tool to guide and refine these species restoration efforts.

Kellie Carim, Aquatic Research Biologist and Tribal Project Coordinator at NGC, has been working on brook trout eradication projects in Montana with Montana Fish, Wildlife, and Parks, and in eastern Washington in collaboration with the Kalispell Tribe and Washington Department of Fish and Wildlife, using a targeted eDNA approach. “Traditional methods can easily detect fish that are present at a population-level density. But they are often not successful at detecting fish at very low densities or in complex habitat,” Carim explains. “The sensitivity of eDNA is allowing us to more confidently determine



Kellie Carim collects eDNA samples in Rattlesnake Creek, Montana, using a pump and filter. A sample can be collected in about 15 minutes and collected samples can be easily stored in the field (photo: M.K. Schwartz, USDA Forest Service).

Tools like eDNA can efficiently remove any guesswork about where the target species is. This can help managers more efficiently mark the area that needs treatment at the beginning of the project, which will reduce the overall treatment area and project costs.

when an eradication project has been 100 percent successful.”

The most effective way to remove invasive trout is to perform a chemical removal, typically used in areas where there are no native species left. “The chemical treatment will basically kill all the fish in the system. It can be a very complete method, but it is expensive, requires a large crew and large volume of chemicals, and can cause impacts to other organism such as invertebrates in the stream,” says Carim. Tools like eDNA can efficiently remove any guesswork about where the target species is. This can help managers more efficiently mark the area that

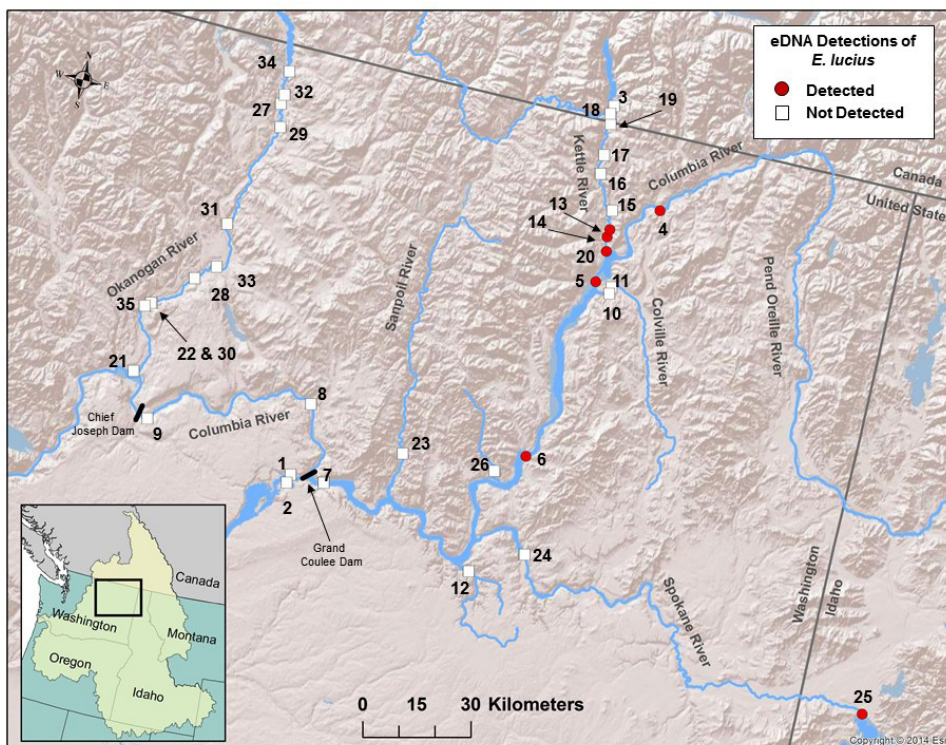
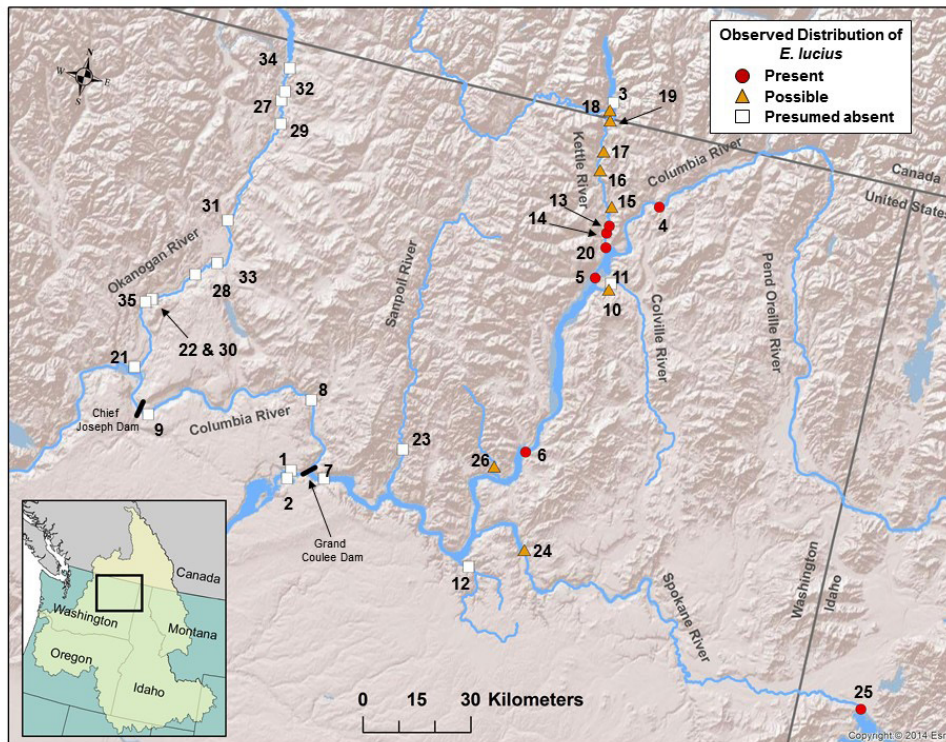
needs treatment at the beginning of the project, which will reduce the overall treatment area and project costs.

Typically, it takes more than one chemical treatment to eradicate brook trout, and managers usually treat the entire system two or three times. “But,” Carim points out, “there are portions of the stream where eradication may have been successful after one treatment. Environmental DNA is so sensitive that it can allow you to identify the areas where the target invasive species persist so again you can reduce your treatment area for those subsequent treatments, further reducing costs

and impacts to the rest of the aquatic community.” Then, at the end of the project, eDNA can help verify project success by a lack of detection of the target species.

Tracking Invasive Species With eDNA: Northern Pike

Carim has also been involved in efforts to track invasions of Northern Pike using eDNA in collaboration with the Confederated Tribes of the Colville Reservation in Washington. At issue is the fact that this invasive species has spread to just upstream of the Grand Coulee Dam on the Columbia River. If pike expand to downstream of the dam, they will be in waters with populations of threatened and endangered salmon. “Once pike get into those areas, the ways in which we can manage them become very complicated,” she explains.



This map shows the high correlation between the observed distribution of northern pike using traditional sampling methods (angler surveys and gill netting) and where they were detected using eDNA in the Columbia River above the Grand Coulee Dam (graphic: K. Carim).

Above the dam, managers can set gill nets—a lethal form of sampling—so the fish can be caught and eliminated at the same time. “Once you’re in waters where you have endangered species, you can’t set gill nets because of the risk of inadvertently killing those endangered species,” says Carim. “The Confederated Tribes of the Colville Reservation is working really hard to raise awareness, get funding, develop suppression efforts, and prevent the spread of the pike. And we’ve been using eDNA in conjunction with some of the conventional sampling like gill netting to locate areas where pike may have spread and monitor areas where they haven’t yet arrived. The Confederated Tribes of the Colville Reservation has been using the results from eDNA to help move up the chain to policy makers and advocate for more policy and funding to prevent the spread of the pike.”

Furthering Our Ecological Understanding: The Future of eDNA

Although NGC is best known for eDNA sampling of fishes, they have also been working on other eDNA frontiers by developing eDNA sampling tools to survey for rare or recovering mammals, water birds, and amphibians. Other groups have designed similar eDNA tools to survey water bodies for invasive wildlife, such as feral swine and pythons.

Many groups working with eDNA are chasing the “holy grail” of

establishing a standard approach to sample all life in a stream simultaneously using nontargeted approaches. “We are at the point with eDNA where we can pull up a sample and we try to detect every single species that’s on that filter,” says Schwartz, “whether it’s a fish, macroinvertebrate, fungus, or bacteria. The problem is that this ‘metagenomics’ approach is not as sensitive and there are both false positives and false negatives. We’re still learning how to use this tool.”

But as these community-level methods are being refined, Schwartz believes that there is a lot of room for the more common, targeted eDNA approaches to further our ecological understanding, beyond just the detection of invasive and rare species. For example, Wilcox, Schwartz, and others completed a study where eDNA was used to determine how the invasive brook trout are influencing the native, threatened bull trout and to develop predictions over time given different climate scenarios—all based on sampling water for eDNA. In other words, species detections from eDNA were used to gain better ecological understandings of how species interact.

Another forthcoming study uses eDNA to refine the distribution models that predict where bull trout would occur across the Columbia River Basin, which would give the managers very fine-scale resolution maps. “Across the entire field,” says Schwartz, “these types

of studies are so rare, but they provide the information that helps managers decide where to best put conservation dollars.”

“These types of studies are so rare, but they provide the information that helps managers decide where to best put conservation dollars.”

How eDNA is Being Used by Managers

- For a USFS restoration project involving vegetation treatments for fuels and road improvements in Twelvemile Creek in the Lolo National Forest in Montana, eDNA sampling is being used to monitor the presence or absence of threatened bull trout. This has saved the USFS time by obviating formal Section 7 consultations with the USFWS on the species.
- Scientists from [NGC](#) have been working on brook trout eradication projects using eDNA sampling in Montana and in eastern Washington with state agencies and tribes to efficiently remove any guesswork about where the target species is and help managers more efficiently mark the area that needs treatment at the beginning of the project.
- Northern pike, an invasive species, has spread to just upstream of the Grand Coulee Dam on the Columbia River. If they expand to downstream of the dam, they will be in waters with populations of threatened and endangered salmon. Genomics Center scientists have been working with the Confederated Tribes of the Colville Reservation in Washington to monitor this species using eDNA sampling.

photo: K. Zarn, USDA Forest Service



Interested in Using eDNA?

What if you are interested in using eDNA for your project—how to begin? NGC makes it easy for land managers to get started with eDNA, with two people whose full-time jobs are to be project coordinators for work with partners. Taylor Wilcox explains, “Someone could just send an email to NGC@usda.gov and explain what they are interested in doing and then NGC scientists would direct it from there. If it's not something routine, we would work with some of the senior scientists here to come up with a research plan.”

Owning the right types of sampling equipment should not be a barrier to getting started with eDNA. “The lab here can provide a lot of resources. For example, if you want to go out and collect samples as a partner, we have a library of equipment here. We can just send you a pump and the sampling equipment you need on a loan basis, and then you can collect samples and then ship all that stuff back. So if our partners are just starting out on this, they don't have to make a big upfront investment,” says Wilcox.



The sampling equipment for eDNA is simple, involving mainly a pump and filter. These can be borrowed from the National Genomics Center lab so that partners can minimize their upfront investment (photo: T. Franklin, USDA Forest Service).

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SCIENTIST AND MANAGER PROFILES

The following individuals were instrumental in the creation of this Bulletin:



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