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Science FINDINGS

INSIDE

"Science affects the way we think together."
Lewis Thomas

The Promise of Environmental DNA: Life in Northwest Streams Revealed Through a Bottle of Water

Brooke Penaluna, a research fisheries biologist with the Pacific Northwest Research Station, collects a water sample from Oak Creek in western Oregon. Environmental DNA is extracted from samples and analyzed in a lab to determine the species that live in and around the stream. Photo courtesy of Ivan Arismendi.

IN SUMMARY

Streams and rivers are a rich repository of minute traces of genetic material from all organisms that live in or near the water, from the tiniest microbes to fish, mammals, and trees. This environmental DNA (eDNA) can tell a detailed story about the life within and around the waterbody.

Brooke Penaluna and Richard Cronn, scientists with USDA Forest Service Pacific Northwest Research Station, have spent the past several years studying the potential uses of eDNA as a tool for understanding what's living in Pacific Northwest streams. Through eDNA analysis, they have identified more than 900 taxa living in Fall Creek in western Oregon. And they were able to identify distinctions within species, giving a more nuanced picture of what's inhabiting Northwest waters. Penaluna also found that the distribution of coastal cutthroat trout in 60 streams in Oregon and Washington was more extensive than previously thought.

Their methods show promise as a survey tool for public and private natural resource managers who are tasked with protecting endangered fish and other species, as well as monitoring the introduction of aquatic invasive species. The current survey method, in use since the 1960s, is electrofishing, which is more labor-intensive, can only be done in wadable water, and is potentially hazardous to stream life.

More work needs to be done to make eDNA analysis an efficient and cost-effective tool in the field, but the possibilities are nearly endless.

"A drop of water,
if it could write out its own history,
would explain the universe to us."

—Lucy Larcom, teacher and author

Twenty-eight population groups of salmon and steelhead on the West Coast are listed as threatened or endangered under the federal Endangered Species Act (ESA). More broadly, the number of total species—from fish to mammals, insects, and birds—that are endangered or threatened is rising dramatically. More than 1,300 species are currently listed under the ESA, up from 281 in 1980.

The USDA Forest Service actively works to conserve wildlife habitat and promote recovery, but to know which species to manage for, federal land managers need data. For fish, that means knowing what species are living in each watershed, how far their populations extend up- and downstream, and the size or density of specific populations.

Until recently, the standard method for collecting this information has been electrofishing. Used since the 1960s, electrofishing involves wading through streams and stunning fish with an electrical device. It's usually done in pairs: one person stuns the fish, the other captures it

Key Findings

- Analyses of environmental DNA (eDNA) collected from streams provide insights into the presence and diversity of aquatic species and how they're affected by forest management.
- A new genomics-based eDNA method identified about 900 taxa of fish, amphibians, arthropods, and pathogens, including native, invasive, endangered, cryptic, and rare species.
- An eDNA survey of coastal cutthroat trout in Washington and Oregon found that fish were present 50 to 250 meters (55 to 273 yards) farther upstream in more than half the sampled waters than previously known. This showed that eDNA testing is more sensitive and thorough than electrofishing, the more traditional method of surveying fish populations.
- A genomics-based method using eDNA samples can be used to noninvasively measure and track genetic diversity of Pacific salmon and trout.

in a dip net. Together, they identify, measure, and release the fish back into the stream. As a census-taking tool, it works, but it can be arduous. It's also potentially harmful to the fish: the electricity causes muscle spasms that, albeit very infrequently, can damage their vertebrae.

Electrofishing can also require more paperwork. In Oregon, for example, depending on the location of the stream, samplers need to have permits from Oregon Department of Fish and Wildlife—one for fish and one for amphibians. If you plan to be in waters with fish that are listed under the ESA, an additional permit is needed.

Another method is gaining interest in the scientific community, thanks to research over the past several years by Brooke Penaluna and Richard Cronn with the USDA Forest Service Pacific Northwest (PNW) Research Station. It involves analyzing stream water for environmental DNA (eDNA), the minute traces of genetic material shed by all living organisms. With eDNA testing, you don't need to shock fish. You don't even need to see them. You just fill a plastic bottle with stream water, filter it, and take it back to the lab for analysis.

Testing eDNA is not only a reliably safe option for fish, it also provides a more complete picture of what is living in the stream system—including fishes, amphibians, mammals, invertebrates, and pathogens. And that picture could change the way land managers protect threatened and endangered species, manage for invasive species, and manage forests.

"If you're a manager and are charged with knowing everything in the environment, electrofishing will only give you part of that information," says Penaluna, a research fisheries biologist.

Exploring the Hidden World

The effectiveness of eDNA testing has been known since the mid 1990s. It has been widely applied to look for single species of animals, fungi, and bacteria in diverse environments. But Penaluna wanted to expand its possibilities. In 2014, she asked Cronn if eDNA could identify multiple distantly related species simultaneously. Cronn, a research geneticist, has worked for the Forest Service for 20 years using DNA testing on a wide variety of applications, from identifying tree parentage to determining the presence of plant pathogens.

"We were used to looking for needles in very big haystacks (or single genes in very big genomes), but there was no on-the-shelf technology to apply to the work Brooke was interested in," Cronn says.

They designed a study in which teams of researchers performed both electrofishing and eDNA sampling at five sites in Fall Creek, a tributary of the Alsea River in western Oregon. They chose this stream because it was near a fish hatchery and had known populations of various fish species.

Electrofishing identified 13 species, including fish, crayfish, and amphibians; eDNA sampling identified all those species and many hundreds more. Penaluna, Cronn, and PNW Research Station biological science technician Laura Hauck adapted a method primarily used in agricultural and medical genetics called microfluidic metabarcoding and applied it to fish, amphibians, and other organisms. They

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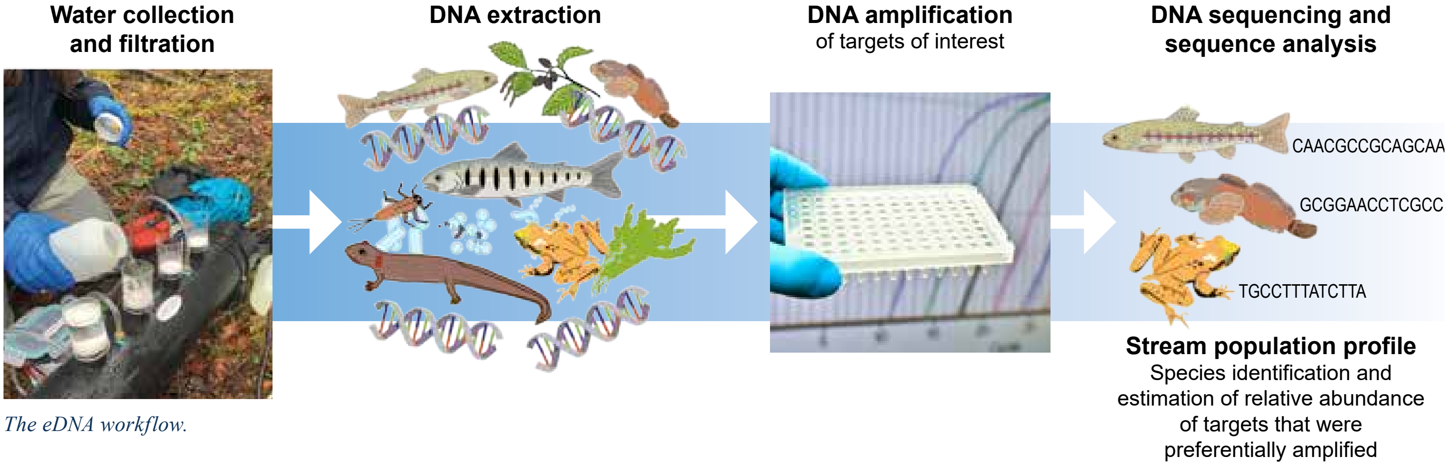
A technician measures a coastal cutthroat trout caught by electrofishing in Mack Creek at H.J. Andrews Experimental Forest, Oregon, before releasing it back to the stream. Photo courtesy of Ivan Arismendi.



The eDNA workflow.

A research team electrofishing. Prior to eDNA analysis, electrofishing was the most efficient method for detecting fish and other aquatic species. Photo courtesy of Lina DiGregorio.

were able to identify about 900 taxa of fish, amphibians, mammals, arthropods, and pathogens through this novel application.

“We could identify a lot of things that were not being surveyed for,” Cronn says. “It was the rest of the hidden world.”

For example, eDNA testing detected beaver and other mammals. It found pathogens responsible for root rots in forest trees—an important early indicator of potential tree disease. Also, unlike electrofishing, it could distinguish individual species of juvenile fish. To the human eye, juvenile rainbow and coastal cutthroat trout are indistinguishable.

The eDNA metabarcoding method proved to be an effective all-in-one census tool for stream communities. Penaluna then used information from eDNA metabarcoding to develop a more targeted approach, focusing only on coastal cutthroat trout (*Oncorhynchus clarkii clarkii*). In particular, she wanted to compare eDNA testing’s effectiveness to that of electrofishing in defining how far upstream cutthroat trout lived in 60 forested streams in Oregon and Washington. She chose coastal cutthroat because the species is known for inhabiting the highest extent of its stream networks, making it an ideal species for using eDNA to determine its upper distribution.

Knowing the upper extent of fish populations is important to forest managers. Stream reaches with fish are managed differently and have more protections from forest harvest practices, such as wider riparian buffers, than streams without fish.

Previous studies showed that eDNA testing is comparable to, or more sensitive than, electrofishing, particularly when species are in low abundance. But it hadn’t been evaluated empirically within the context of forest management by identifying the boundary line between “fish” and “no fish” in a stream network.

“Our objective was to understand the relative reliability of eDNA as a management tool to detect the upper extent of fish,” Penaluna says.

Penaluna and her research team collected water in 2017 and 2018. They did it from March to June to coincide with the recognized sampling window for evaluating the “end-of-fish” boundary under the forest practices rules for each state. This is considered the optimal sampling window because it is when fish are thought to be farthest upstream. It’s also when water levels begin to drop, making it easier for sampling crews to work.

The crews collected water samples every 50 meters (55 yards), starting 175 meters (191 yards) downstream from the last known end-of-fish boundary (determined by previous electrofishing surveys), and worked their way upstream, ultimately going beyond the boundary. After they collected eDNA samples, they electrofished the area, then compared the results.

They found that in more than half the streams sampled, the end-of-fish boundary was anywhere from 50 to 250 meters (55 to 273 yards) upstream from the electrofishing boundary.

“The implications are that protections for forests should extend to where the fish are,” Penaluna says. “Our research shows that, currently, that extent is underestimated.”

The state of Oregon is now using the new eDNA-based estimates as it considers the size of buffers for private landowners. And other scientists with the Forest Service, National Park System, and the nonprofit conservation group Trout Unlimited are using this method to evaluate the upper extent of fish in stream and river reaches.

Both studies—the metabarcoding study that looked at all organisms, and the cutthroat study that looked at single species—illustrate the power of eDNA sampling.

“Streams work as accumulators of DNA. They’re also transporters. Any given water sample contains DNA samples from the nearby trees, algae, bacteria—everything from primary producers to the largest carnivores,” Cronn says. “So the potential complexity of the water is the sum of all the genomes in the forest.”

Focusing on Biological Diversity

With proof that eDNA analysis can effectively identify both the breadth of species in a stream system and the habitat extent of specific species, Penaluna and Cronn worked with Kevin Weitemier, a postdoctoral scholar, to take it a step further. They wanted to see if eDNA could be used to identify the genetic diversity within species.

Information on genetic diversity gives fishery managers valuable insights into the magnitude and distribution of heritable genetic variation in different populations. This variation affects how fish can adapt to environmental extremes, how susceptible they are to disease, how affected they are by barriers to migration and reproduction, and other factors that influence their survivability. Because genetic variation is heritable through the passage of DNA from parent to offspring, it is also a measure of future population resilience to environmental change due to climate or management activities.

Laura Hauck, a biological science technician with the Pacific Northwest Research Station, works with eDNA samples in the Corvallis Forestry Sciences Laboratory. Photo by Rich Cronn, USDA Forest Service.

For this study, the scientists focused on multiple species of Pacific salmon and trout, which are among the most intensively managed wild species in North America.

They collected and analyzed water samples from 15 sites in Oregon and 1 in adjacent northern California using the metabarcoding method they developed previously. The tool did an excellent job in picking up small genetic nuances within the fish populations, providing a detailed view of the variations of salmon and trout within the sampled sites.

In some cases, their findings corresponded with previous studies in which investigators took small fin clips from fish caught by electrofishing. But with eDNA, the sampling was easier and potentially less harmful to the fish.

"We picked up all the major genetic variation characterized in earlier studies of collected fish, and we did it just by filtering water," Cronn says. "That's one of the real advantages of eDNA testing. It avoids the harassment of fish, especially where they're endangered."

That said, currently, no single method produces perfect detection. Although eDNA sampling provides a wealth of information about stream life, there are some questions that are still better answered through electrofishing.

"eDNA doesn't tell me the size or weight of the fishes, so I don't know if they are juveniles or adults. I don't know the parasite load on the fishes. I know parasites are there, but not necessarily which fish species are infected. I also don't know deformities in the fishes from things like poor water quality," explains Penaluna.

Private Industry Takes Notice

Ashley Coble, a forest watershed scientist with the National Council for Air and Stream Improvement, Inc. (NCASI), has worked with Penaluna and Cronn. In a study published in 2019, they documented the potential for eDNA in evaluating the effects of logging roads on aquatic species, assessing the effectiveness of culverts and other constructed fish passages. They also discussed how eDNA could be used to monitor the wastewater treatment systems of lumber mills.

By conducting eDNA sampling, researchers identified this pool as the farthest reach for coastal cutthroat trout on Nevergo Creek in the Willamette River basin in western Oregon. Photo by Brooke Penaluna, USDA Forest Service.

"Private industry has been very engaged in pushing this research forward to incorporate forestry applications," Coble says. "It's still a novel technology, which means it's going to take years for the potential to be fully realized."

Measuring the full range of biota in a stream system is expensive, requires a specialized lab, and takes a lot of time—all of which are roadblocks to its use by industry. But the more narrowly targeted approaches, such as measuring for a single fish species as a way to define end-of-fish boundaries, is more feasible, according to Coble. In fact, portable field instruments the size of a cell phone can now sequence eDNA in remote settings.

"This is likely to make eDNA studies more flexible, affordable, and powerful," Coble says.

Weyerhaeuser, the largest private timberland owner in the United States, is one of the private companies taking an interest in eDNA. The company has used eDNA in the Southeastern United States to test its effectiveness in surveying freshwater mussels. Weyerhaeuser is also dabbling with eDNA in the Pacific Northwest.

"We want to be on the forefront of new methods," says Jessica Homyack, a wildlife biologist and Weyerhaeuser's director of environmental research and operational support.

Homyack worked with Coble, Penaluna, and Cronn on the 2019 study, and one of her staff members, Jason Walter, coauthored the report on Penaluna's end-of-fish boundary study of cutthroat trout. Weyerhaeuser is partnering with Penaluna on a project in Oregon where they're using eDNA to see how aquatic species are using streams in areas affected by wildfire.

Weyerhaeuser currently still uses electrofishing to survey fish boundaries. But Homyack says eDNA analysis holds a lot of promise, especially in its ability to help scientists understand the biodiversity of stream systems.

"The technology is improving rapidly," she says. "We're not quite ready to switch over; there are still unknowns and lots to figure out. But we're excited to be involved and we're following the science."

"Research is formalized curiosity. It's poking and prying with a purpose.

—Zora Neale Hurston, author

Writer's Profile

John Kirkland has been writing about science, higher education, and business for more than 20 years. He lives in Portland, Oregon.

LAND MANAGEMENT IMPLICATIONS

- Analyzing water samples for environmental DNA (eDNA) is a noninvasive method that reveals species distributions, can be used to measure population genetic diversity, and helps resource managers meet multiple management objectives related to aquatic biodiversity.
- Information gleaned from eDNA to determine the upper extent of fish in a stream can be used by resource managers to make more informed decisions and provide optimal protection for aquatic species of concern.
- Genomics-based eDNA approaches provide science-based, data-driven information that empowers managers and policymakers to make more inclusive conservation decisions based on evidence from multiple species.

For Further Reading

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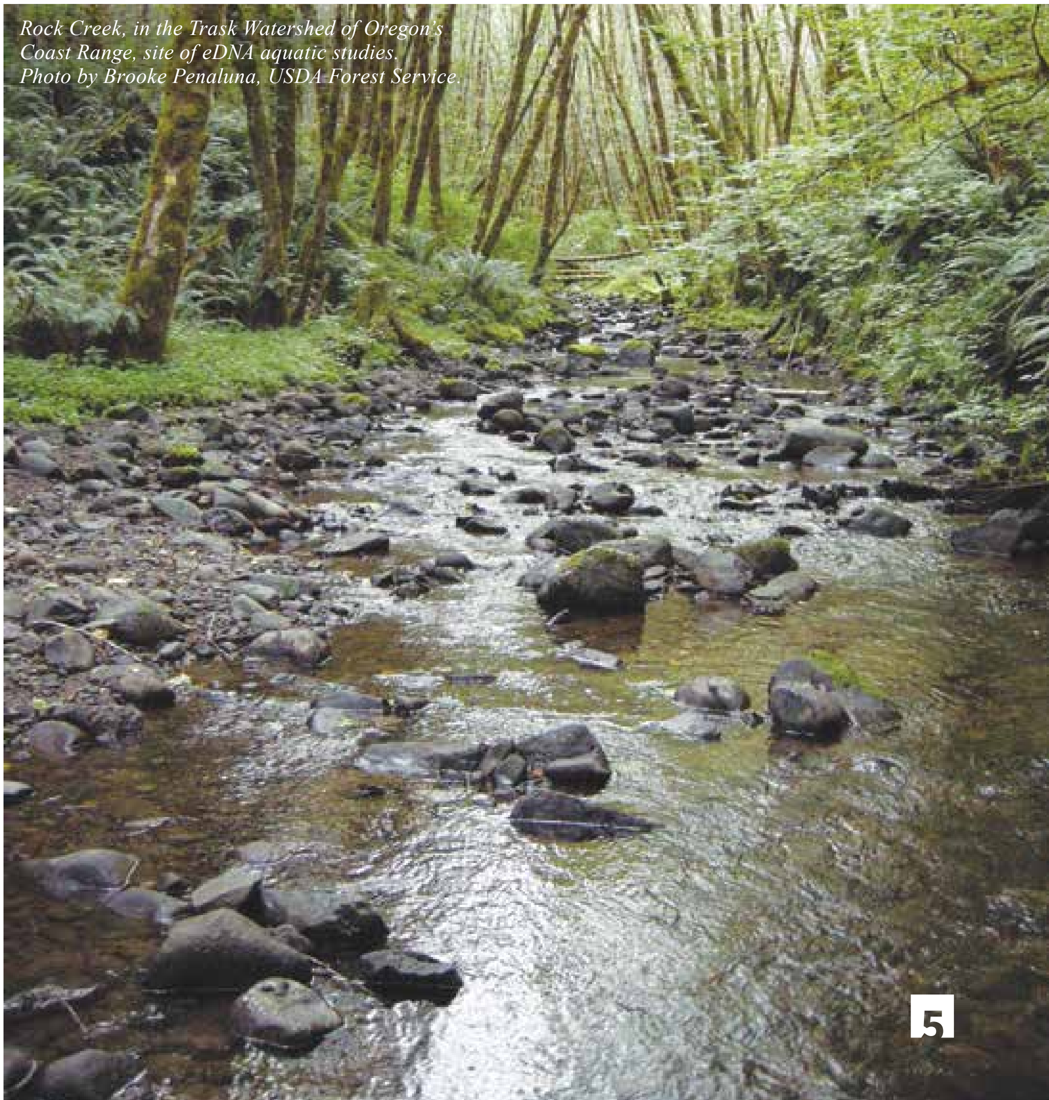

Rock Creek, in the Trask Watershed of Oregon's Coast Range, site of eDNA aquatic studies. Photo by Brooke Penaluna, USDA Forest Service.

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Scientist Profiles

BROOKE PENALUNA is a research fisheries biologist whose work focuses on understanding the effects of climate change, forest harvest, and other disturbances on fish in both riparian and aquatic habitats. She was recently named the lead scientist at the USDA Forest Service's H.J. Andrews Experimental Forest, a 16,000-acre site in Oregon's western Cascade Range dedicated to the research of forests, streams, and watersheds. Penaluna holds a Ph.D. in fisheries science from Oregon State University.

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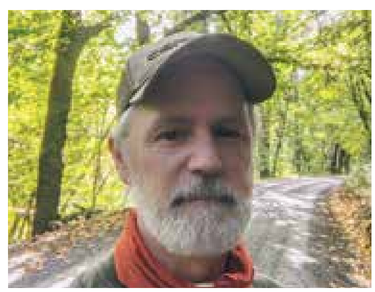

RICHARD CRONN is a research geneticist with more than 20 years of experience applying genetic research to forestry science. His current research includes advancing genetic marker technologies for trees to help prosecute timber theft; linking genetic differences in conifers to the environmental pressures that influence them; and adopting genomics technologies such as eDNA for forest management. Cronn holds a Ph.D. in plant genetics from Iowa State University.

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