Monitoring river health using a robotic water sampler

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In 2018, an Environmental Sample Processor was installed at a US Geological Survey streamgage station (lower left) on the banks of the Yellowstone River. Credit: Jim Birch/MBARI

Researchers from MBARI and the US Geological Survey (USGS) recently published a paper showing several ways that MBARI's Environmental Sample Processors (ESPs) can be used to monitor the health of rivers. The ESPs, which are essentially robotic laboratories, were used to collect and preserve samples of water from the Yellowstone and Snake Rivers. By analyzing "environmental DNA" in the river water, the researchers were able to detect introduced and invasive animals as well as microbes that can cause disease in humans and fish.

Environmental DNA (eDNA) is DNA that is released by organisms into their environment in the form of bits of skin, mucus, or bodily waste. In the case of aquatic organisms, this DNA may be detectable in the surrounding water for as long as several days. The goal of this research, as described in the journal *Scientific Reports*, was to compare eDNA from water samples automatically collected by ESPs with eDNA from water samples manually collected by humans.

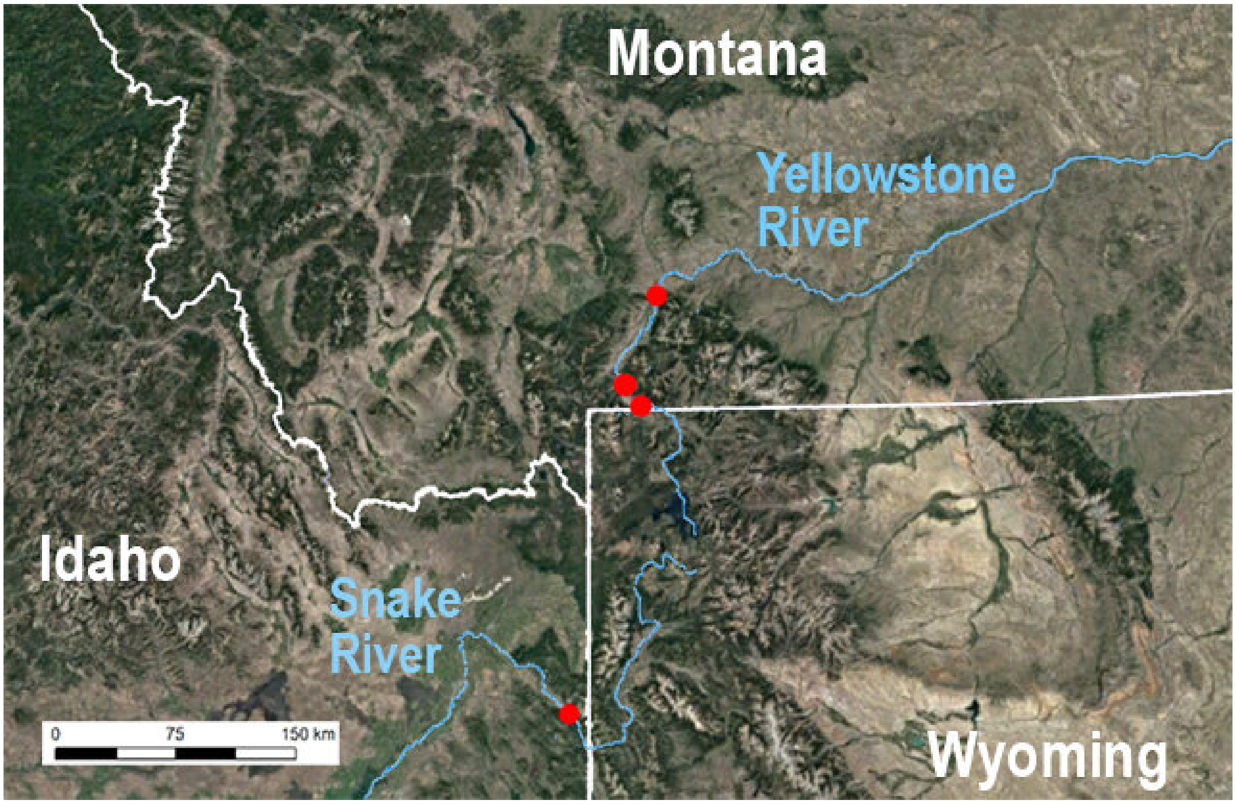
The water samples were collected at several different times and places. In 2017, a pilot sampling program was conducted at the confluence of the Boiling and Gardner Rivers in Yellowstone National Park. This was followed by more extensive sampling programs at three sites on the upper reaches of the Yellowstone and Snake Rivers in 2018 and 2019.

In analyzing the water samples from these sites, the researchers looked for eDNA from several different organisms:

- Protozoans in the genus Naegleria: Sometimes known as the "brain-eating amoeba," this microbe can cause deadly brain inflammation in humans. During the pilot study, DNA from Naegleria protozoans was detected in all the samples collected by the ESP as well as in hand-collected samples. Fortunately for people swimming in the sampled rivers, infections from these organisms are relatively rare.
- Tetracapsuloides bryosalmonae: A microscopic relative of jellyfish, this organism causes kidney disease in trout and salmon, and had previously caused an outbreak at one of the study sites. During this study, eDNA from this organism was detected only occasionally, but it appeared in both the ESP- and

hand-collected water samples.

- Chub mackerel (Scomber japonicas): This marine fish is not naturally present in rivers, but was used to simulate the presence of an invasive species. The researchers placed several kilograms of canned mackerel into the water upstream of two sampling sites to see if mackerel DNA would be detected in their lab tests. Once again, mackerel DNA was found in both the hand-collected and ESP-collected samples.
- Freshwater mussels in the genus Dreissena: This invasive species is found in many freshwater streams, but was not known to be present at the sites used in this study. In an encouraging finding, neither the ESP- nor the hand-collected water samples contained DNA from these mussels.
- Sockeye salmon (Oncorhynchus nerka): This sportfish was intentionally introduced into the Yellowstone and Snake rivers but is rarely found during fish surveys. During this study, sockeye salmon DNA was detected sporadically by both sampling methods. The large numbers of samples collected by the ESP were particularly useful in detecting this elusive fish.



The red dots on this map show the locations of USGS streamgage stations where Environmental Sample Processors were used to study river health. Credit: Base map: Google Earth.

As the examples above show, the water samples collected by the ESPs yielded similar eDNA results as did the samples collected by USGS technicians. ESP sampling also reduced the cost, time, and risks involved in repeatedly sending technicians out to remote locations. As the authors of the study put it, "ESPs can conduct high-frequency sampling, regardless of location, weather or the availability of human resources."

The ESPs used in this study can collect samples automatically every three hours for at least three weeks. Frequent sampling is key to detecting subtle changes in river health. For example, when an area is just beginning to be colonized by an invasive organism, there are relatively few individuals present, so their eDNA is widely dispersed and not well mixed with the surrounding water. This means that researchers need to collect lots of samples to reliably detect the early stages of an outbreak.

The large numbers of samples collected by the ESPs gave biologists more definitive information about whether invasive species were present or absent. As the authors explained, "a negative ESP result provides some confidence that the target species DNA is absent, while a 'no data' result from less-frequent manual sampling provides no [useful] information."

"This is one of the more important findings of this study," said MBARI Research Specialist Kevan Yamahara. "We are always looking for positive detections of species of interest. However, the non-detection of species of interest is essential for this kind of work, and can only be done confidently using high-frequency sampling such as the ESP provides."

This photo shows an Environmental Sample Processor (large gray cylinder), along with filters and pumps inside a USGS streamgage station. Credit: Jim Birch/MBARI

Although the ESPs worked well overall, Yamahara noted that the MBARI team faced challenges that they had not encountered when operating ESPs in the ocean. "During one of the deployments," he said, "The river stage dropped significantly and we were no longer able to pump water. Another challenge was air temperature. It gets cold in the fall in Idaho and Montana and we had to use some tricks to keep the ESP warm while the temperature outside dropped below freezing."

Both Yamahara and the paper's lead author, Adam Sepulveda, credit former MBARI Senior Research Technician Roman Marin III for addressing these challenges and making the deployment a success. As Yamahara put it, "Roman did a lot of the heavy lifting on this project." Roman passed away in 2019, but his legacy continues in the form of research projects such as this one.

Identifying and solving these kinds of challenges will be critical if the USGS goes ahead with its ambitious plans to install monitoring systems at streamgage sites around the country. The USGS presently maintains over 8,200 of these sites, which provide real-time data about the amount of water flowing in rivers and streams. Adding instruments that can monitor river health would be a logical extension of this network.

The authors of the paper note that because ESPs are still relatively expensive (about $100,000 each for the models used in this study), they are most cost-effective at remote stream sites. However, they add, "The full potential of robotic technologies like the ESP will be realized when they can execute in situ analyses of water samples and transmit results to decision-makers."

Roman Marin III inspects one of the Environmental Sample Processors used by the USGS. Credit: Jim Birch/2018

MBARI researchers are currently working on this, testing a variety of automated sensors that could be hooked up to an ESP to detect eDNA automatically. At the same time, Yamahara is busy modifying and improving MBARI's ESPs so they can collect and prepare samples for such automated sensors. "A lot of our focus right now is on sample acquisition and preparation for in situ analysis," he said. "I think we need to be confident we have robust methods for supplying good samples to analyze. That said, we are constantly investigating new technologies for in situ analysis as well."

Such automated monitoring systems, if they can be made inexpensive enough, would yield the "holy grail" of biological monitoring—near real-time data that resource managers can use to study river health, detect problems, and fix them before they get out of hand.

More information: Adam J. Sepulveda et al. Robotic environmental DNA bio-surveillance of freshwater health, *Scientific Reports* (2020). DOI: 10.1038/s41598-020-71304-3

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