Environmental DNA provides early detection of invasive crayfish

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Every plant and animal has a unique genetic composition, which makes a lake like a bowl of DNA soup—every spoonful contains the combined DNA of the lake's inhabitants. Scientists have only recently begun using this environmental DNA, or eDNA, to identify the presence of organisms like amphibians and fish. A U of I researcher and his colleagues analyzed eDNA to successfully detect the presence of the highly invasive rusty crayfish in a dozen Wisconsin lakes. Using eDNA to monitor hard to detect species can provide early warnings of newly arrived invasive species.

"The lakes in the Boulder Junction area have had long-term monitoring from the University of Wisconsin and the University of Notre Dame, so we had an existing gradient of lakes where this invasive crayfish had never been observed to lakes where we know rusty crayfish are abundant," says U of I aquatic ecologist Eric Larson. "Using the eDNA tool we succeeded in detecting rusty crayfish in lakes where this species is very rare. This suggests that the tool could be used to monitor for early warning of new invasions in other regions, which would let us enact control or eradication measures when they're most feasible."

Larson says he was skeptical of using eDNA for this particular species. The tool has been successful in finding fish and amphibians which are very mobile, more mucousy, and, presumably, constantly shedding DNA into the environment. "With my background as a field biologist, I thought—Crayfish. With an exoskeleton. Under a rock. At the bottom of a lake. I didn't think we'd find any using this environmental DNA approach. Obviously, I'm a convert."

In the study, samples were analyzed using a small white machine that could be easily mistaken for a bread maker. Inside, a computer with a laser heats and cools the samples of DNA over and over in a chemical solution. During each cycle, the double strands of DNA are separated, then built up again. The duplication is exponential so millions of copies are created within a very short time. Beforehand, a dye is attached to the DNA, making it easier for researchers to identify each organism's DNA and quantify it.

Larson's colleague Mark Davis, coordinator of the Collaborative Ecological Genetics Laboratory at the Illinois Natural History Survey, explains that every living thing is constantly sloughing off cells and all of those cells contain DNA. But eDNA isn't like what you get if you take a blood sample from a salamander. That would be "clean DNA." You already know it's from a salamander.

"The eDNA from a lake is 'dirty' DNA," Davis says. "It's degraded, broken down so you have very small fragments and few copies. With chemistry and technology, we amplify it. Using bioinformatics, the computer wades through the information to give us a full complex of what's in that sample—whether it be invertebrates, fish, reptiles, amphibians, birds—anything that may be coming into contact with the water or soil. With eDNA, it's exciting because you don't know what you'll find."

Davis says there are still eDNA problems to solve. "Right now we can tell if an organism is present or not. But knowing the exact number of individuals is difficult. For example, we often don't know the rate an organism sheds DNA or if they shed more at different times. How quickly does it degrade?"

Larson says that one potential disadvantage to using this hypersensitive tool is that it may increase the potential for finding false positives, or cases where an organism is perceived as present when it's not. This can occur if field or laboratory equipment is contaminated or if DNA is transported long distances via predators or water currents. In the case of Larson's study, crayfish eDNA was detected in two lakes where the invader had not previously been observed by more conventional methods. Larson says that a minute amount of

DNA could have been transported in feces from birds that had fed on crayfish in a different lake, as one example of potential error associated with eDNA.

"It may be that these are new or incipient invasions that eDNA detected before other methods. But it may also be that we had false positives. As a consequence, these are lakes that we want to monitor and follow-up on," he says.

Globally, there are around 600 crayfish species, of which only about a half dozen have become problematic invaders in the United States. These non-native crayfish prey on fish eggs and destroy aquatic plants, and can negatively affect fish through competition for food and changes to their habitat.

"There are economic repercussions from invasions," Larson says. "One eradication of rusty crayfish in Wisconsin took years and was very costly." In that instance, success may have been due to a drought that substantially lowered the lake levels and stranded their habitat.

"Crayfish can walk over land so if you have them in an aquaculture pond there's nothing to prevent them from crossing over a little hill and then showing up in a national park," Larson says. "They're also prevalent in elementary and middle school science classrooms as live animals for behavioral studies. Teachers may not want to euthanize the crayfish at the end of the school year. Often believing that there is just one crayfish species everywhere, they have an end-of-semester release party and dump aquarium contents into a local pond or stream, or send crayfish home with students who may subsequently release them."

Larson says preventing invasions from happening in the first place is ideal. "But the eDNA tool gives us a sensitive and potentially affordable method for monitoring hard to detect species for management applications. That can mean early warnings of these species invasions while you still have the time to control or contain them before they are too abundant for that to be feasible."

More information: Matthew M. Dougherty et al. Environmental DNA (eDNA) detects the invasive rusty crayfishat low abundances, *Journal of Applied Ecology* (2016). DOI: 10.1111/1365-2664.12621

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