

## Using environmental DNA for to survey the populations of endangered species

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R. a . suigensis is an endangered species facing local extinction in their natural habitats in Japan. Scientists at Okayama University have developed a novel environmental DNA survey method that can aid conservation efforts for this species. Credit: Kazuyoshi Nakata



Changes in river systems, overfishing and the appearance of new, invasive species can lead to a drastic decline in the number of native fish inhabiting aquatic ecosystems. In the Ashida river basin in Japan, the bitterling fish (Rhodeus atremius suigensis), which was previously found in abundance, is now facing local extinction. This is concerning, considering that it is recognized as an indicator species for the conservation of fish diversity in freshwater ecosystems.

Conservation efforts to protect native aquatic fauna require field studies of large areas to understand the habitat needs and population density of different species. This is a challenging task, requiring substantial time and effort. To overcome this obstacle, scientists usually focus on small areas and trace the DNA discarded by living organisms into their environment. This environmental DNA (or eDNA) can be analyzed to identify species that recently visited the area, in a non-invasive and timeefficient manner.

Recently, scientists from the Okayama University in Japan used eDNA to survey not only the presence but also the distribution and population density of R. a. suigensis in the Ashida river basin in Fukuyama, Japan. They employed a semi-quantitative eDNA analysis method using the polymerase chain reaction (PCR) technique.

They developed DNA primers that detected a stretch of the mitochondrial DNA of R. a. suigensis, which made their analysis highly specific to only this particular species. Their findings were published in *Landscape and Ecological Engineering*.

"We first confirmed the utility of this analysis in aquarium experiments, before performing field surveys," says Prof. Kazuyoshi Nakata from Okayama University, who led the study. "We set fish traps at 48 points



in an agricultural channel in the Ashida river basin and examined the relationship between fish presence and eDNA concentration."

The research team also included Ms. Kanoko Otsuki, Dr. Mayuko Hamada, and Prof. Tatsuya Sakamoto from Okayama University, and Dr. Noriyuki Koizumi from the National Agriculture and Food Research Organization.

The researchers found that eDNA concentrations vary according to the downstream channel distance from the point where specimens of R. a. suigensis were captured—the greater the distance, the lower the eDNA concentration. "Our results serve as a reference to how far and how much downstream eDNA can be detected, which will be useful to guide future conservation surveys," says Prof. Nakata.

Thus, the researchers could verify that the concentrations of eDNA were indicative of the distribution and abundance of R. a. suigensis. Since this technique requires only the sampling of water in the field, even local residents can help carry out ecological surveys. Future <u>conservation</u> <u>efforts</u> can apply the information obtained from these surveys to design appropriate strategies.

This technique is highly scalable and can be replicated for larger areas. Further, with the development of corresponding molecular tools, such as specific primers, this technique can be modified to survey other <u>endangered species</u> as well. This will help in not only the promotion of the conservation of endangered species, but also contribute invaluably towards awareness regarding the importance of biodiversity conservation with the involvement of local communities.

**More information:** Kanoko Otsuki et al, Quantitative PCR method to detect an extremely endangered bitterling fish (Rhodeus atremius suigensis) using environmental DNA, *Landscape and Ecological* 



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