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New environmental DNA method detects invasive fish species in river water

Scientists have developed a new way of monitoring Ponto-Caspian gobies, a group of widely invasive fish species, by detecting traces of the fishes' DNA in river water. They say, in a recent research paper, that it offers a quicker, easier and cheaper way of monitoring the fish than conventional catching or sighting methods.

Ponto-Caspian gobies are a group of five small invasive fish species, native to the Black and Caspian Seas, which have colonised freshwater and brackish waters in Europe and North America. They have spread via ballast water released from ships and are typically introduced in harbours before swimming up rivers. One of the species, the round goby (*Neogobius melanostomus*), is among the 100 worst invasive species in Europe, according to the DAISIE database.

Current catch and sighting methods for monitoring Ponto-Caspian gobies are labour-intensive, expensive, need specialist skills and are not very sensitive. These species are particularly difficult to monitor because they hide under stones, and appear first at sites, such as harbours, that are difficult to monitor by conventional approaches.

Environmental DNA (eDNA) analyses, or 'assays', are an emerging and increasingly popular technique for monitoring biodiversity, particularly species that are hard to detect using conventional methods. They detect the presence of a species from traces of DNA that it has left behind in the environment (from their cells, for example), and they are already available for a number of species[1].

The researchers demonstrated how they used their eDNA assay for Ponto-Caspian gobies on water taken from eleven sites along the Rhine River, in and around the city of Basel, Switzerland. These were all locations for boating and shipping activity, such as harbours and marinas.

They collected the water using a modified version of a commercially available water sampler. This captures water from the bottom layers of the river where DNA concentrations for Ponto-Caspian gobies (which live on the riverbed) were assumed to be greatest, as well as from surface water. As the researchers suspected, goby eDNA concentrations were higher in deeper layers of the water (4 m deep), which highlights the importance of considering species lifestyles when developing eDNA assays. The water sampler was decontaminated after each visit to a site, to avoid cross-contamination, as this could potentially and incorrectly indicate the fishes' presence at subsequent goby-free sites.

Water was taken at different times of the week, as the researchers believed that eDNA concentrations could be affected by ships (active on weekdays) stirring up the water. However, the results were the same for samples taken on a Wednesday and those taken on a Sunday.

They analysed the water in the laboratory, looking for short strands of goby DNA. As part of this study, the team established primers that are specific to two species — the round goby (which is known to be heavily present in the area), and the bighead goby (*Ponticola kessleri*), as well as a primer for all five of the invasive Ponto-Caspian gobies. They made sure that these could not be confused with DNA from other goby or native Swiss fish species.

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Source: Adrian-Kalchhauser, I., Burkhardt-Holm, P. (2016). An eDNA Assay to Monitor a Globally Invasive Fish Species from Flowing Freshwater. *PLoS ONE* 11(1): e0147558. DOI :10.1371/journal.pone.0147558. This study is free to view at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0147558.

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1. Thomsen, P.F. & Willerslev, E. (2015) Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation* 183: 4–18. DOI: 10.1016/j.biocon.2014.11.019. www.sciencedirect.com/science/article/pii/S0006320714004443

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The DNA fragments were amplified using PCR (polymerase chain reaction). PCR is a method of repeatedly copying a small piece of DNA to build up a large enough sample for analysis. It is often inhibited by substances in the environment, such as humic acids released by decaying leaves. To prevent this interference, the researchers added a substance called Bovine Serum Albumin (BSA) to the water samples.

Only round goby eDNA was detected, and was found in water from five of the 11 river sites; these were the sites closest to a harbour which is densely populated by this species. The researchers cannot be sure that no gobies were present in the remaining six sites further along the river, as they do not know the limits of the assay's sensitivity. To optimize results, water could be collected during larval drift periods; juvenile and adult fish are bottom-dwelling but larvae drift into the water column and are, therefore, more likely to be included in the water sample, providing additional biological material, and thus detectable DNA, during the extraction. The researchers have also identified ways in which the laboratory assay itself could be made more sensitive.

The eDNA assay is not suitable for assessing abundance, the researchers say. They compared its data with catch data, and found that more eDNA had been detected at a site where fewer fish had been caught, compared with a site where more fish were caught.
This new technique for detecting Ponto-Caspian gobies is simple and needs less specialist expertise than conventional monitoring methods, the researchers say. It can, therefore, enable environmental managers to map invaded areas more easily than at present.