Environmental DNA (eDNA) and monitoring for invasive species

What is eDNA and how is it used for dreissenid mussel early detection by RDLES?

Environmental DNA (eDNA) refers to DNA present in an environmental sample, as differentiated from traditional sampling of DNA directly from an intact organism. eDNA frequently is thought of as DNA in tissue and cells that have been shed by an organism, but can also refer to DNA within an intact organism (usually microscopic), if that organism is collected in the environmental sample. For eDNA analysis, samples are collected from the environment and DNA is then extracted from the full sample or some fraction of it. To determine whether an organism of interest is present, a polymerase chain reaction (PCR) is performed with PCR primers that are specific to that organism. This reaction will amplify millions of copies of a fragment of DNA only from the species of interest. eDNA assays allow surveillance for the presence of an organism in an environment without having to collect the whole organism itself. eDNA detected by this approach can come from a wide range of sources, including feces, mucus, fish scales, and hair, among others. Because the purified eDNA is a mixture representing multiple species and individuals present in the environment, this technique can be used to detect a wide range of organisms, including those that are endangered or invasive, and be used for both research and monitoring purposes. Over the last few years the use of eDNA has expanded and there is currently a wide range of active research ongoing to expand our knowledge of how this approach compares to traditional biological survey methods.

There are both advantages and limitations to the use of eDNA. It provides a "snap shot" of the environment at the time of the sample collection and gives evidence that the organism of interest is present. It can detect organisms that are at low abundance, and at any life stage or gender. Also, it enables researchers to collect samples with little disturbance to the environment. Many samples can be collected and the cost of sample analysis is comparatively low. However, because of differences in the source organism's ecology and physiology, eDNA is not distributed evenly in the environment. Due to this, a "negative" eDNA PCR result should not be taken as direct evidence that the organism of interest is absent from the sampled environment. As with any other survey technique, such a failure to detect the organism may indeed mean it is not present, or it may be that the organism is present, but its scarcity or distribution are such that it was not detected. In addition, eDNA analysis does not give an estimate of the number of organisms of interest present in the environment, nor does it indicate if the organism was dead or alive when the sample was collected.

How does RDLES use eDNA?

The Reclamation Detection Laboratory for Exotic Species (RDLES) makes use of eDNA techniques to analyze water samples from across the western United States for the presence of quagga and zebra mussels.

- Water samples are collected using the plankton tow method, and are buffered with sodium bicarbonate and preserved with alcohol.
- Once the samples arrive at the RDLES laboratory they are analyzed with cross polarized light microscopy (CPLM) for the presence of quagga and zebra mussel veligers.

- If a veliger is found in a sample the veliger is removed, and both the veliger and the bulk sample are PCR assayed independently for the presence of quagga or zebra mussel specific DNA. The water body will then be placed on a "priority" list and all subsequent samples will be subject to both CPLM and eDNA PCR analysis.
- In the RDLES laboratory, "priority" samples first undergo CPLM analysis, and then DNA is extracted from the bulk water sample using a commercial DNA extraction kit. PCR is performed on this eDNA purification to detect the presence of invasive mussels.
- In addition, when suspect veligers are found in a sample, they are isolated to be photographed and then undergo DNA extracted and PCR to identify the species. PCR primers from the cytochrome oxidase I (COI) gene that are specific to either quagga mussel or zebra mussel are used. COI is a mitochondrial gene with multiple copies in each cell.
- Following the PCR reaction, the resulting PCR products are analyzed by gel electrophoresis, to determine whether a positive PCR product has been produced by the reaction. The results are accepted if both the positive and negative controls pass QA/QC.
- The PCR reaction is considered positive if a single band is observed in the gel electrophoresis that corresponds in size to the positive control band (~380 base pairs). All positive PCR products are sent to a commercial laboratory for DNA sequencing to confirm that they are from either quagga or zebra mussel. The sequenced DNA must have at least 90% identity to a reference quagga or zebra mussel COI gene sequence to be considered positive.
- Because of the low concentration of mussel specific DNA present in eDNA samples or individual veligers, there are instances when a PCR assay is determined to be positive, but there is not sufficient product for DNA sequencing. In these cases, DNA sequencing results is considered "Non-sequenceable". Once these tests are completed, the results are reported to our clients.

RDLES provides PCR results for both the bulk water samples (eDNA) and for any suspect veligers that are found in the water samples. A positive eDNA PCR result from the bulk water sample indicates that dreissenid mussel tissue was present in the sample. Although this result may not occur with a veliger body for all samples, it can inform water managers that dreissenid mussels may be present in their water and additional monitoring should be conducted.

Alternatively, a positive PCR result from a veliger body indicates the veliger collected from the sample contained tissue and was positively identified as either quagga or zebra mussel. A negative PCR result from a veliger body is generally due to a lack of sufficient tissue in the veliger to confirm it was dreissenid. It does not definitively mean that the veliger is not dreissenid. In the absence of a positive PCR result the taxonomy of the suspect should be utilized to confirm identification. The RDLES lab utilizes multiple tests to increase the likelihood that the presence of dreissenid mussel is detected.