RECLAMATION
Managing Water in the West

U.S. Department of the Interior
Bureau of Reclamation

Research Update

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Bottom Line

Early detection of quagga and zebra mussel veligers is difficult because the organisms are microscopic and sensitive to degradation from both water chemistry and organic matter. This research study determined the most effective way to preserve samples to maintain veliger integrity over time.

Better, Faster, Cheaper

Detecting mussels at the onset of colonization alerts facility managers early and allows time to enact control measures. However, proper sample preservation is critical for accurate and early detection.

Correctly preserved (top left) versus incorrectly preserved (right) quagga mussel veligers after 21 days in solution.

Impact of Sample Preservation on Detection of Invasive Mussels

How sample preservation impacts the early detection of invasive mussels by microscopy and polymerase chain reaction

Problem

Invasive *dreissenid* mussels disrupt facilities and greatly increase operation and maintenance costs. These invasive quagga and zebra mussels also affect the overall ecology of a reservoir by filtering large amounts of water and decreasing the zooplankton populations that fish and other animals need to survive. Eventually, these shifts in ecology can cause algal blooms and proliferation of weeds.

The Reclamation Detection Laboratory for Exotic Species (RDLES) has been successfully monitoring for the early detection of quagga and zebra mussel veligers (larvae) in the Western United States since 2007. This monitoring effort alerts facility managers at the onset of mussel colonization, allowing time to enact control measures.

Early detection of veligers includes identifying veliger bodies via microscopy and/or detecting small amounts of free-floating DNA via polymerase chain reaction (PCR). Both the veliger body and the DNA are sensitive to degradation. If the veligers shell or tissue is degraded, it will not be detected. Therefore, it is important to maintain the integrity of samples after collection. Mussel shells are composed of calcium carbonate, and acids can degrade these shells. Acids can be introduced, either from surrounding organic materials in the reservoir water (inhibitors) or from preservatives (alcohol). Ineffective sampling methods could result in unnoticed mussel infestations, which can delay treatment and containment.

Developing a standard operating procedure (SOP) for early detection sample preservation is a consistent, effective method that allows Reclamation and its partners the best opportunity to spot mussel invasions early.

Solution

This Reclamation Science and Technology Program research study project built on previous studies to help determine the best preservation method for early detection samples analyzed by microscopy and PCR. Unlike previous studies, this research determined how samples containing additional organic material and reservoir water affect veliger degradation. This research study tested the impact of eight sample preservation scenarios on veliger detection by microscopy and PCR. The preservation scenarios tested combinations of variables, including presence or absence of alcohol, presence or absence of buffer, and high or low levels of zooplankton (inhibitors). Five replicates of each preservation scenario were prepared in a controlled laboratory setting. Veliger shell and DNA degradation were assessed after veligers had been preserved for 1, 6, 21, and 42 days.

Application and Results

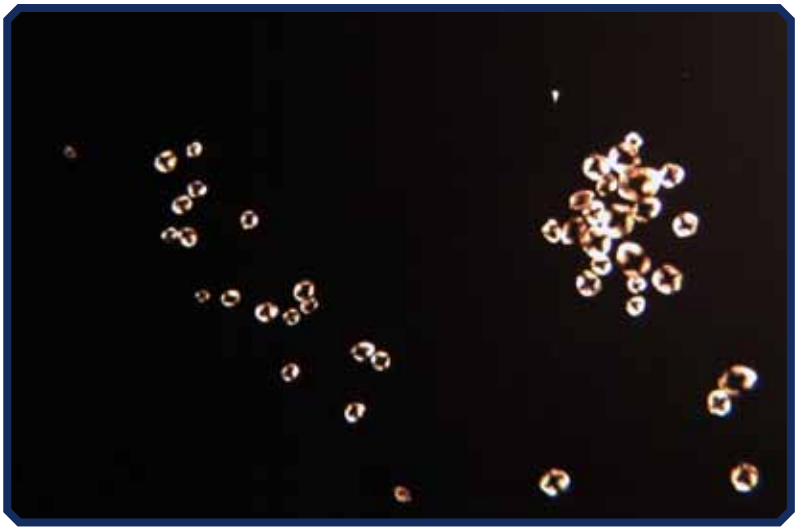
The results of this research study suggest that regardless of alcohol content, veliger detection by microscopy is reduced as samples age and when samples are not buffered and contain high inhibitors. Veliger shell morphology can be maintained for 42 days after collection if samples are preserved with 20 percent alcohol per volume and buffered with 0.02 gram per liter of baking soda. PCR detection of veligers was also best when samples were preserved with both alcohol and buffer but, overall, detection was reduced as holding time increased. The results of this research study indicate that correct sample preservation is critical to maintain veliger integrity over time, especially because both water chemistry and organic content appear to impact veliger degradation.

Future Plans

Reclamation's mussel detection program involves several agencies who collaborate to sample multiple water bodies across the Western United States. Because this program has so many collaborators, sample collection and preservation methods are not always uniform.

These results benefit Reclamation and RDLES by explaining how veliger morphology and detectability are impacted by improper preservation in "real world" water samples over time. The results of this study and previous studies (Carmon et al., 2014) have been used to develop an SOP for field collection of quagga and zebra mussel veligers.

Reclamation needs to be able to present this data to its collaborators for the consequences of improper sample preservation to be fully understood and appreciated.



Mussel veligers under cross polarized light microscopy.

More Information

www.usbr.gov/research/projects/detail.cfm?id=3157

Carmon, J. and D. Hosler. 2013. Field Protocol: *Field Preparation of Water Samples for Dreissenid Veliger Detection*. Field Standard Operating Procedure, Version 4. Technical Memorandum No. 86-68220-13-01. Bureau of Reclamation. www.usbr.gov/mussels/docs/FieldSOPPreparationandAnalysis.pdf

Carmon, J., J.A. Keele, S.F. Pucherelli, and D. Hosler. 2014. "Effects of buffer and isopropanol alcohol concentration on detection of quagga mussel (Dreissena bugensis) birefringence and DNA." Management of Biological Invasions, 5(2):151-157.

"Early detection of invasive mussels is the only way to protect our waters from devastating infestations, but it is like looking for a needle in a haystack. Proper sample preservation greatly increases sample integrity and increases the likelihood of detecting the 'needle'."

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For printable version see: www.usbr.gov/research/docs/updates/2015-13-sample-preservation.pdf