

Research Update

Fall 2015
Bulletin 2015-16

Bottom Line

Dreissenid mussel management efforts designed to limit or prevent secondary invasions and damage to water delivery infrastructure depend on accurate monitoring and efficient information dissemination. An important component of detection monitoring programs is the ability to accurately and reliably detect, as early as possible, the introduction of these mussels into a pristine water body.

Better, Faster, Cheaper

Developing the most reliable and sensitive method of detection for the presence of *dreissenid* mussels will help safeguard Reclamation water delivery infrastructure, with the goal of early mitigation of costly operations and maintenance issues before they occur.

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Monoclonal Antibodies for Improved Detection of *Dreissenid* Mussel Larvae

A novel method for the early detection of quagga and zebra mussels

Problem

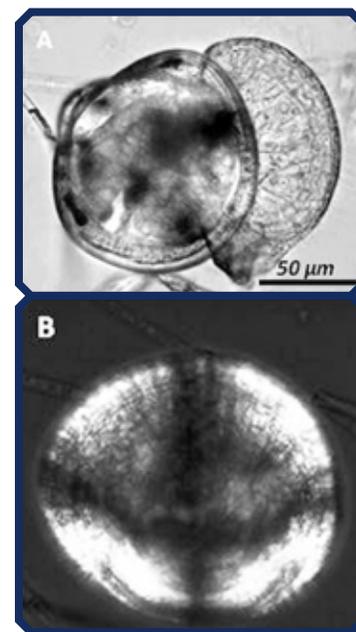
The expansion of *dreissenid* mussels into the Western United States has generated an increased need for reliable early detection methods, especially for larvae (veligers), which are a primary transport vector and an indicator of spawning adult mussels. Following initial introduction into an aquatic system, mussels attach to most submerged surfaces, resulting in serious consequences for the drinking water and hydroelectric power industries, industrial cooling facilities, agricultural irrigation, and recreational use of water. Colonies of mussels clog intake trashracks, pipes, valves, siphons, and irrigation and fire suppression systems. Consequently, it is critically important to detect infestation in the early stages so that timely and cost effective response plans and control strategies can be developed.

The mussel life cycle includes microscopic, planktonic larval stages (veligers) that are typically detected using cross polarized light microscopy on plankton net concentrates. However, other plankton species and inert materials also produce similar results and can be confused for quagga or zebra mussel veligers. In addition, concentrated water samples usually contain many other organisms and debris that can interfere with veliger detection. Therefore, tools and methods are needed to simplify and improve veliger detection to ensure maximum confidence in the results of monitoring programs.

During a nationwide double-blind, round-robin study involving 19 independent laboratories, available detection methods were found to generate both false positive and false negative errors, even in relatively clean water samples. Federal and State authorities have called for standardized *dreissenid* monitoring or quality assurance standards, and there is a growing consensus that laboratory certification and quality assurance programs would be useful for management communities responsible for monitoring and mitigating new invasions and spread of *dreissenid* mussels.

Solution

This Reclamation Science and Technology Program research project developed monoclonal and polyclonal antibodies (mAb, pAb) that can be used to label veligers with fluorescent tags and purify veligers from complex samples using magnetic capture technology. Organism-specific antibodies have previously been used to improve detection and identification of many organisms in environmental waters, including marine mussel larvae and human pathogens. An antibody that recognizes quagga and zebra mussel veligers, coupled to fluorescent tags or magnetic beads, would greatly improve detection and identification of veligers in aquatic systems.



Quagga mussel veligers (umbonal life stage) viewed by conventional microscopy. (A) White light microscopy image with extended vellum (B) Cross polarized light microscopy image.

Application and Results

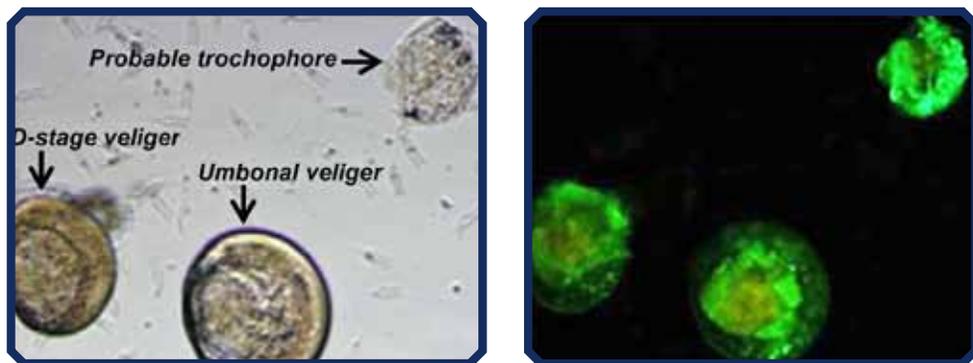
These antibodies will be used to purify veligers from complex environmental water samples using magnetic capture technology and to label veligers with fluorescent tags. Fluorescently labeled organisms are much easier to detect and enumerate by microscopy. Organism-specific antibodies have been used in many fields to aid in isolating organisms from complex samples and to label those organisms with fluorescent tags, thus aiding detection and identification. For example, the U.S. Environmental Protection Agency's approved method, which is used nationwide for detecting the protozoan parasites *Cryptosporidium* and *Giardia* in water, uses antibodies for immunomagnetic purification and detection by fluorescence microscopy. Of more direct relevance to the current project, monoclonal antibodies have been used to detect and identify larvae of the economically important mussel, *Mytilus galloprovincialis*. Sensitive molecular methods have been developed to detect quagga and zebra mussel veligers and other invasive mussels. Compared to conventional microscopy-based methods, they are most useful as early warning monitoring tools, while direct observation by conventional microscopy remains the most appropriate approach for monitoring sites that are already infested and assessing the level of infestation.

The goal of this research project was to produce a mAb that selectively binds to quagga mussel veligers. A variety of mAbs were produced as unpurified, laboratory-scale preparations and as purified, reagent-grade reagents in milligram quantities. These antibodies stained veligers, generating a variety of fluorescence staining patterns when observed by indirect immunofluorescence microscopy. The project successfully demonstrated the feasibility of generating mAbs that recognize and bind to quagga mussel veligers. The antibodies produced by this project provide tools that could simplify detection and identification of veligers in water samples.

Future Plans

Additional specificity testing is necessary, along with testing the performance of mAbs against veligers in more complex matrices. Continued development of the immunocapture technique is also required. All of the antibody-producing cell lines developed for this project are stored as frozen (-80 °C) stocks. These frozen cell stocks can be used to generate additional purified antibody for further research efforts in collaboration with interested stakeholders and research institutions.

Additional work to further develop and refine these antibody-based veliger detection tools is recommended: 1) evaluate staining patterns and intensity with all larval stages of quagga mussels (D-shaped, umbonal, and pediveligers); 2) thoroughly evaluate specificity and quantification of false positives and false negatives with a variety of nontarget organisms; 3) improve reduction of autofluorescence; 4) evaluate different sized paramagnetic beads, including <100 nm beads; 5) assess alternative secondary bridges linked to magnetic beads to improve magnetic capture with mAbs; and 6) identify and characterize the protein antigen(s) recognized by the antibodies using protein separation by polyacrylamide gel electrophoresis and Western blotting.



D-stage and umbonal veligers and a possible trochophore stained with cell culture supernatant from a hybridoma subclone. White light microscopy (left) and epifluorescence microscopy (right).

“Quagga mussels are a huge issue for us in the Lower Colorado Region. We have reservoirs with quagga mussels, and those without that we really would like to keep that way. Therefore, finding new ways to detect mussels early is critical to us in protecting our water infrastructure. This research will help us do just that.”

Nathaniel Gee
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More information

www.usbr.gov/research/projects/detail.cfm?id=9640

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