Early detection of dreissenid species: Zebra/Quagga mussels in water systems

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Abstract

Early detection of dreissenid species in water systems is critically important to maintaining structure and function of dam related structures. If mussels are detected early, facility operators may have approximately three to five years to adjust systems before the population of mussels are large enough to restrict the flow of water, clog pipes, restrict water intakes, affect cooling systems, and impede power generation. However, early detection of dreissenids in water with the current methods has some inherent issues with variability in sampling and reliability with analytical methods which creates management decision dilemmas. Since current control methods of these mussels are very limited in regulated water systems, Reclamation is conducting intensive research. To improve early detection in water systems, Reclamation has developed an analytical strategy that includes cross polarized light microscopy, scanning electron microscopy (SEM), flow cell cytometry, and polymerase chain reaction (PCR). This analytical strategy is used on water samples collected using a plankton tow net to collect primarily from reservoirs and also from water flow within facilities, usually from a tap or pipe.

Key words: Early detection, dreissenid species, zebra mussels, quagga mussels, cross polarized light microscopy, scanning electron microscopy, flow cell cytometry, polymerase chain reaction, SEM, PCR, Reclamation

Introduction

The dreissenid species have the ability to rapidly attach and colonize hard surfaces producing dense stacks of mussels upon any hard surface, which results in serious economic problems for recreation and facility managers. Dreissenid mussels are major biofouling organisms that can clog water intake structures (such as pipes and screens), reducing pumping capabilities for power and water treatment plants, at high cost to industries, companies, and communities. Recreation-based industries and activities have also been impacted (docks, breakwalls, buoys, boats, and beaches have all been heavily colonized) (MacIsaac 1996; Mackie et al. 1989).

The life cycle of the dreissenid mussel progresses from a microscopic phase (veliger) to a thumbnail sized adult. The larval phase impacts the food web and fisheries, while adults also affect the food web and fisheries; and they impact the structure and function of water delivery systems by impeding flow, clogging pipes, and increasing corrosion (Caludi and Mackie 1998; U.S. Army Corps of Engineers 2008).

Early detection of the presence of dreissenid mussels can be made by finding adults in the system, generally with the use of substrate samplers, or by the collection of the larval stage of the mussels in water samples using plankton tow nets (Johnson 1995). Field experience at Reclamation indicates that for early detection, plankton tow net samples are the best method to detect mussel presence.
Identification of the two adult dreissenid species currently found in the United States can be differentiated by morphological differences of the shell.

The adult zebra mussel \textit{(Dreissena polymorpha (Pallas, 1771))} has a definite angle, or carina, between the ventral and dorsal surfaces, whereas, the adult quagga mussel \textit{(Dreissena rostriformis bugensis Andrusov, 1897)} has a rounded carina. The ventral side of the zebra mussel shell is flattened, but the quagga mussel has a convex ventral side. This characteristic can be distinguished by placing shells on their ventral side and generally, the zebra mussel will remain upright whereas a quagga mussel will topple over. Overall, quagga mussels are rounder in shape and zebra mussels are more triangular (Bureau of Reclamation 2008). With the use of a light microscope, the dreissenid veliger can be distinguished and identified by its characteristic shape, size, and cross pattern appearance under cross polarized light. Differentiation between the two species in the larval stages is more difficult as the taxonomic characteristics can slightly overlap (Johnson 1995; Nichols et al. 1994; Bureau of Reclamation 2008; U.S. Army Core of Engineers 2000, 2008).

Dreissenid colonization may have adverse impacts on the structure and function of the dam and associated facilities. Early detection of dreissenids allows facility operators to implement corrective action to mitigate the impacts of the mussels before the facility structure and function is severely affected (Bureau of Reclamation 2008). While adult zebra mussels are fairly easy to distinguish from other mussel species, identification of the immature veligers is more difficult and studies that consider only abundances of older shelled stages (visible by cross-polarized lighting) may also yield misleading results. There is high variability associated with sampling that is attributed to environmental, seasonal, and identification factors. The mean relative percent differences ranged from 8-12 percent in laboratory duplicates and from 15-22 percent in field sample duplicates (Stefanik 2004).

Initially, Reclamation developed an early detection program for zebra and quagga mussel larvae collected in plankton tow net samples (Allen 1997). It was designed to be a two-pronged process involving the visual microscopic diagnostic test followed by a confirmatory DNA test. The results between the two methods did not agree in all cases. One source for this discrepancy in results can occur due to potential misidentification of an organism due to similar looking organisms present in waters, primarily ostracods and Asian clams (Johnson 1995; Nichols et al. 1994). The sheer volume of DNA present in the plankton tow samples coupled with a developing technology for the genetic identification of dreissenids in raw water samples can also explain some of the inconsistencies.

In an effort to improve testing agreement, Reclamation developed and employed improved microscopic methods (using a microscope with cross-polarized light source) for detecting veligers; and improved polymerase chain reaction (PCR) methods for detecting zebra/quagga mussels through the amplification of trace DNA in plankton tow samples. Additionally, Reclamation expanded its research to include improved sample collection, preparation, and additional analytical equipment. While the consistencies in analytical results have improved, the research has provided answers to some of the reasons for variability in test results. The addition of SEM provides clear photographic records that assist the expert microscopists in identification of organisms demonstrating birefringence under cross polarized light (Baldwin et al. 1994; Martel et al. 1995).

While conducting dye studies utilizing flow cell cytometry, photographs demonstrated that in dyed preserved samples, the euthanized veligers exhibited a separation of veliger shell and tissue. This observation provided an explanation as to why pipetted veligers may not reveal a positive with PCR testing. If the microscopist was pipetting a shell without its tissue into a PCR test tube, then amplifiable DNA was not present in the sample yielding a negative result. Additionally, preservative testing demonstrated that the pH of a water sample and concentrations of ethanol greater then 10% dramatically affected the shelf life of the organism itself.

Methods

The accepted diagnostic test for the presence of dreissenid veligers is cross-polarized microscopy, which may then be confirmed by the presence of dreissenid DNA using PCR. Errors in identification by the microscopist may be further confounded by the negative results of the PCR tests. Originally, Reclamation utilized the Army Corps of Engineers (USACE 2000)
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Figure 1. Examples of Sample preparation, microscopy and a PCR gel plate used in the early detection methods for dreissenid mussels. Photos courtesy of U.S. Bureau of Reclamation.

Figure 2. Dreissenid mussel veligers by Cross Polarized Light Microscopy Left: Zebra mussel veligers, Right: Quagga mussel veligers. Photograph by Denise Hosler, U.S. Bureau of Reclamation.

method for veliger enumeration, however the method was modified as the concentration of veligers in Lake Mead became too numerous to count. The modified procedure combined Standard Method 10200G (Standard Methods 2001), the US EPA Method LG403, Revision 2 (USEPA 2003), and the USACE procedure which yielded consistent quantitative results with the continued benefit of early detection when veliger counts were below five veligers.

The sample preparation was modified to allow veligers to settle in an Imhoff cone for a minimum of 24 hours and the results of this modification led to a 98% recovery rate of veligers in the first 15 mLs off of the cone. This recovery rate was determined by analyzing successive 15 mL aliquots of the settled sample and examining the contents of each 1 mL aliquot under cross polarized light microscopy. Previous testing of veliger detection utilizing sucrose filtration indicated improved recovery rates, therefore sucrose filtration was tested in the settling cones, however the recovery rate was lower at 87% (Claudi pers. communication; Schaner 1990). Therefore, the continued use of the sucrose filtration method in the cones was abandoned. Per the USACE method, the mean of five cell counts is used to obtain the mean number of veligers per milliliter in the sample.

In the event of discovering a suspect positive by microscopy, the addition of SEM photography was found to clearly identify the suspect organism. By pipetting the suspect organism onto a glass slide and preparing it for SEM microscopy, it was found that the subsequent photographs improved accurate identification. In some cases, cross polarized light microscope photographs made it difficult to differentiate ostracods, Asian clams, dinoflagellates, and gastropods from dreissenid mussels. Under cross polarized light the dreissenid veliger is more rounded and has a clear right angle cross compared to the ostracods. The "D" shape Asian clam is much larger than the "D" phase veliger,
Figure 3. SEM and cross polarized light microscope photographs demonstrating improved diagnostic capability. Upper left: Quagga mussel, Upper right: SEM photo of an Asian clam with a cracked shell next to the cross polarized image of the organism which only slightly larger than a normal “D” phase veliger. Middle Right: SEM photo of an ostracod next to a cross polarized image of a suspect “D” phase veliger. Bottom Row left to right: SEM photos of dinoflagellates and gastropods that looked suspicious to the microscopist. Photographs by Denise Hosler and Doug Hurcomb, U.S. Bureau of Reclamation.

Figure 4. Photographs from flow cell cytometry clearly demonstrating the separation of the mussel tissue from the veliger shell in dyed, preserved samples. The negative control samples were preserved prior to addition of dye and did not reveal loosely attached tissue. The enlarged images on the right display more detail of the trailing tissue associated with veligers that were introduced into the viability stains prior to ethanol preservation. Photographs courtesy of the U.S. Bureau of Reclamation.

and generally has a slight hump on the line of the "D", with a less than perfect cross. That being said, the magnification and taxonomic anomalies can make the definitive identification difficult. The use of SEM improved the diagnostic capability to differentiate a variety of organisms present and resolved questions about the analyst’s identification (Baldwin et al. 1994; Martel et al. 1995). Currently, Reclamation routinely uses SEM for all suspect organisms, which allows for the remaining portion of the sample to be analyzed by PCR for the presence of dreissenid DNA. This new stepwise methodology for accurate, early detection has proven to be quite helpful to those responsible for high dollar management decisions.

Additionally, while conducting veliger viability studies, Reclamation incorporated the use of flow cell cytometry. The water sample is run through a flow cell while a particle analyzer triggers digital photography of each particle entering the flow cell. The subsequent photographs can then be utilized for identification and enumeration. While conducting this research, the technicians at Reclamation began utilizing various viability dyes and stains in an attempt to determine viability at the time of capture. These stains included Rhodamine, neutral red, and nontoxic food dye. The results of this testing revealed that tissue and shell separation in euthanized veligers could be one of the explanations for discrepancies in the results.
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Figure 5. Quagga mussels settling on a trash rack (upper plate), water intake screen (middle plate) and a penstock gate (lower plate). Photos Courtesy of U.S. Bureau of Reclamation.

**Dreissenid impacts:** Various studies have shown that early detection of dreissenids in water systems provides a three to five year window before the populations explode to become a real threat to structure and function. Thus, the importance of identifying the presence of dreissenid species early on, and then assessing the risk potential for infestation and vulnerable locations in the facility, so that management can make changes and adjustments, that will hopefully reduce the economic impacts of an infestation (Claudi and Prescott 2007; Claudi and Mackie 1998; U.S. Bureau of Reclamation 2008).

Typically the dreissenid mussels affect dam structures in two major ways: 1) flow obstruction and/or blockage of conduits and 2) corrosion of metallic and concrete surfaces. At the onset of an infestation, the primary concern is the former. Each dam requires a review of the dam facility to determine the structural locations that may be vulnerable to impacts by dreissenid mussels (It must be prefaced that vulnerable areas are identified only as points of possible risk to adverse impact by dreissenids based on past experiences with infestations elsewhere). Also, if the environmental conditions are not suitable, an invasive population burst may not occur. A risk assessment of the environmental conditions can indicate the likelihood of an infestation; however, there are many locations where infestations have occurred in unfavorable conditions (Cohen 2007; Kennedy 2007). Speculation cannot be made as to how serious the infestations may become or if the function of the dam components will be affected without the gathering of detailed hydrological, environmental and biological information (Claudi and Prescott 2007; Claudi and Mackie 1998; Lucy 2006).

**Conclusions**

The presence of large quagga and zebra mussel populations may have significant impacts on the environment and water-related structures. The economic and functional impacts of an infestation may be overwhelming for water managers (Lovell and Stone 2005; Pimentel et al. 2005). Based upon the historic experience at Reclamation with various testing methods for early detection, substrate samplers which allow mussels to settle on a hard surface, and shoreline surveys have been less effective than plankton tow net sampling. In the absence of finding adult...
mussels, early detection of veligers with plankton tow samples offers management some advanced warning to adjust and modify systems, and also plan operation and maintenance activities to reduce negative consequences. Since early detection methods may occasionally yield conflicting results, it creates a serious dilemma for management decisions that require large dollar expenditures. In the situation where optical and DNA testing do not agree, additional testing utilizing SEM has provided supplementary photographic evidence for accurate reporting. Improved sample handling, monitoring pH, and increasing ethanol concentration to 70% has improved veliger shelf life and PCR results. Experience with DNA testing to date indicates that the larval population must reach higher density thresholds than with microscopic methods for detection and consistent results. When DNA tests reveal a positive result for dreissenids, then the environmental risk assessment becomes the next important management step. Evaluating the environmental conditions gives management some idea of the likelihood of infestation or population explosion. A comprehensive risk assessment of suitability for survival and reproduction, as well as an evaluation of vulnerable points in the dam facility will allow management to prioritize and budget for the necessary activities to minimize dreissenid impacts.

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