

EXECUTIVE SUMMARY

Reliability of Early Detection of *Dreissena* spp. Larvae by Cross Polarized Light Microscopy, Image Flow Cytometry, and Polymerase Chain Reaction Assays

Results of a Community Double-Blind Round Robin Study (Round Robin Study Phase II)

By

Marc E. Frischer¹, Sandra A. Nierzwicki-Bauer², and Kevin L. Kelly³



¹ U.S. Department of the Interior
Bureau of Reclamation
Technical Service Center
Denver, Colorado



Skidaway Institute
of Oceanography

² Skidaway Institute of
Oceanography
10 Ocean Science Circle
Savannah, Georgia



Darrin Fresh Water Institute
A Research Center of Rensselaer Polytechnic Institute

³ Darrin Fresh Water Institute
5060 Lake Shore Drive
Bolton Landing, New York

A double-blind study was undertaken to assess the current reliability of three different methods for detecting *Dreissena* spp. mussel larvae (veligers) in plankton net tow samples. The three methods examined were cross polarizing light microscopy (CPLM), imaging flow cytometry (IFC), and DNA-based polymerase chain reaction (PCR) assays. Reference samples consisting of concentrated plankton spiked with known numbers of *Dreissena* spp. larvae (0-27 range) were distributed to practicing analytical laboratories for analysis. For practical reasons, sampling was conducted in the winter of 2010, although it was recognized that it would have been more realistic to sample in warmer months typical of the periods when most routine monitoring occurs. Eighteen independent laboratories participated in the study and analyzed 216 reference samples. Results indicated that CPLM was the most reliable of the methods, with an overall accuracy for presence/absence detection of 96.3%. IFC analysis was the second most reliable method, exhibiting a 91.7% accuracy rate. PCR was the least reliable method, exhibiting a 75.8% accuracy rate. The most prevalent type of error associated with all of the methods was false negatives, suggesting that all methods are more likely to fail to detect the presence of *Dreissena* spp. larvae rather than to falsely indicate their presence. Of the two microscopy-based methods (CPLM and IFC), IFC systematically underestimated the quantity of larvae in samples, while CPLM was more reliable with respect to larvae quantification. PCR methods are not yet quantitative, so they could not be evaluated in this regard. Based on this study, 10 specific recommendations for improving the reliability of dreissenid early detection monitoring programs are proposed.

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