BY BENJAMIN W. SPAULDING

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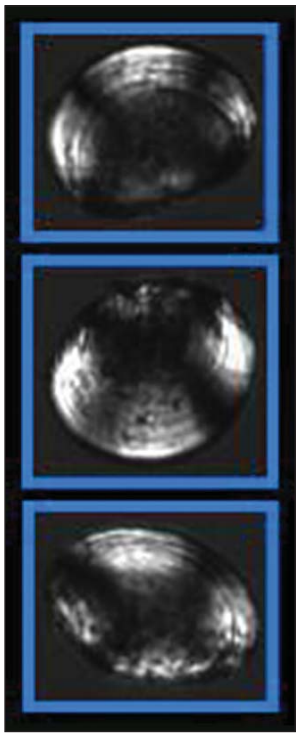
Early Detection Can Help Eradicate Invasive Mussels



Under the cross-polarized light, particles that exhibit birefringence are clearly visible.

Many freshwater lakes and rivers are under seige by invasive aquatic species. These "invaders" can be introduced into water bodies by ballast water from ships, on the hulls of boats (both commercial and recreational), or by any means through which water from one source is exchanged with another. The introduction of these invasive species into an ecosystem can cause major environmental damage and significant financial expense.

Of particular concern in the United States is the presence of invasive mussel species (most notably zebra and quagga mussels) in the Great Lakes and their rapid spread to other bodies of water. Sampling for the larval stage (veligers) is the preferred method for detecting this species. Detection of the veliger at low concentrations indicates the presence of the species, when it is possible to take preventive action. Unfortunately, veligers are small (usually 50–300 µm), and they can only be seen through a microscope. The typically low concentrations commonly necessitate analysis of a large volume of water via microscope or polymerase chain reaction analysis, requiring a significant investment in both time and labor.

In the experiment reported here, the FlowCAM®-XPL was used to detect and count veligers in five 40-mL water samples. This equipment uses birefringence (a double-refraction phenomenon in which an unpolarized beam of light is divided into two beams with different directions and relative velocities) to provide a fast and efficient means of detecting veligers in various concentrations.

MINIMAL OPERATOR TIME REQUIRED FOR MONITORING A SAMPLE RUN

The FlowCAM-XPL uses two cross-polarizing filters to detect veligers. One filter is fixed; the other adjustable (Figure 1). A size-exclusion filtration step uses synthetic nylon screen[1] to remove excessively large particles (i.e., > 300 µm) from the samples to prevent clogging. The variable-flow peristaltic pump used in this process produced a flow rate of approximately 0.5 mL/min of sample, resulting in an average run time of 90 min per 40-mL sample. Because the process is automatic, it isn't necessary for an operator to monitor the sample run.

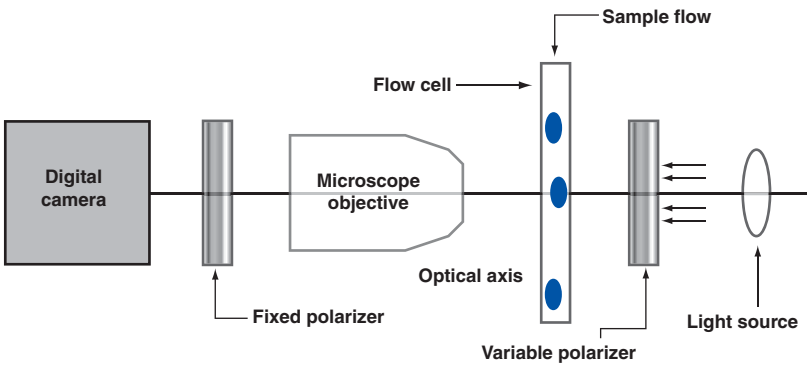
Zebra mussels belong to the *Dreissena* spp., an invasive aquatic species spreading rapidly throughout freshwater lakes and rivers.

CUSTOM IMAGE FILTERS INCREASE EFFICIENCY

Particles that exhibited birefringence were clearly visible. Their typical appearance is a black-and-white shape displaying a distinctive cross pattern on a gray background (see the photograph on page 19). These particles are captured using the VisualSpreadsheet™ image-processing software. The standard image-processing tools available in VisualSpreadsheet can be used to remove any captured images that are clearly not veligers. Development and implementation of custom image filters can also increase the efficiency of this process by reducing the overall data set.

A final manual review of the software filter results was conducted. Particles that were obviously not veligers or other particles of noninterest were removed, even though the particles might have exhibited some birefringence. Fragments and pieces of veliger shells were also removed from the data set. Double images of unique particles were removed at this point in the process. Partial images were visually analyzed to determine whether sufficient morphological information was available to categorize them into the proper grouping. For the experiment described here, the groupings consisted of either *Dreissena* spp. or other particles. The "other particles" group contained organisms that appeared to be similar to *Dreissena* veligers, but because of their birefringence or morphology may have been a different species or an ostracod (a small crustacean). Once the sample had undergone the postprocessing step, the final results were tabulated.

FIGURE 1 Schematic of the FlowCAM®-XPL



CONCLUSION

The FlowCAM-XPL produced results consistent with the actual concentration ranges as calculated by the US Department of the Interior Bureau of Reclamation for the zebra mussel veligers in all five of the unknown samples. In addition, the use of the FlowCAM-XPL and cross-polarized light allowed for completion of this experiment in less than 8 hours with minimal operator attention. Being able to quickly and efficiently analyze water samples for invasive species allows detection of very small veligers at very low concentrations, avoids long delays or preservation methods that may negatively affect veliger concentrations, and frees operator time.

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FOOTNOTES

[1]Nitex screen, The Wildlife Supply Co., Buffalo, N.Y.