A Comprehensive Report on the Workshops "*Dreissena* Early Detection Best Practices" and "*Dreissena* Early Detection Laboratory Standards"

February 7-10, 2012 (Fort Worth, Texas)

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Executive Summary

In 2010, the Quagga-Zebra Mussel Action Plan for Western U.S. Waters (QZAP) was developed by the Western Regional Panel on Aquatic Nuisance Species. One of the highest QZAP priorities identified was the expansion of early detection monitoring programs in the Western region. Expansion of early detection programs depends critically on early detection methodologies and the availability of reliable analytical laboratories to complete timely analysis of plankton samples. With support from the U.S. Fish and Wildlife Service, via competitive QZAP funding (FWS-94140-001), two workshops were conducted that address both of these interdependent requirements. The workshops took place on the campus of Texas Christian University in Fort Worth, Texas, February 7-10, 2012.

The first workshop, presented on February 7-8, 2012, focused on best analytical practices and the identification of quantitative *Dreissena* larvae (veliger) detection limits for the available three primary analytical methods: (1) cross polarized light microscopy (CPLM), (2) image flow cytometry (IFC), and (3) polymerase chain reaction (PCR)-based detection assays. The second workshop, presented on February 9-10, examined the need for a laboratory accreditation program or a laboratory personnel certification program and the development of a roadmap for creating these programs in the Western region.

The workshops attracted 42 participants from 26 different agencies and institutions. The majority of participants were technical experts, but there was strong representation from management and stakeholder groups as well.

To achieve the goals of the first workshop, overview presentations of the state-of-the art methods for each detection approach were provided by recognized experts. Updates of the most recent technological advances in IFC and PCR technology were also provided. In addition, the results from two recent case studies where method comparisons were possible were also presented. Breakout groups discussed the development of Standard Operating Procedures (SOPs) for each method. In plenary discussion, it was decided that SOPs will be written for each method. However, it was agreed that it was not possible to develop a single SOP. Rather, SOPs should be based on equivalency to defined detection limits. It was decided that current best practices should be capable of achieving a detection limit of 10-50 veligers per cubic meter (0.01-0.05 l-1), regardless of the method. In addition to these discussions, several recommendations based on the Round Robin II study (www.musselmonitoring.com/RRII_Recommendations) were discussed and prioritized for the purpose of recommending further steps to improve *Dreissena* spp. early detection capabilities. The top three priorities were: (1) improved PCR-based detection technology, (2) establishment of laboratory certification standards, and (3) utilization of CPLM as the primary approach for early detection of *Dreissena* spp. larvae. A complete

list of priorities can be found at the mussel monitoring Web site: www.MusselMonitoring.com/Ranked_Priorities.

To achieve the goals of the second workshop, descriptions of existing laboratory certification and training programs were presented by experts from the perspective of the public health field, a commercial laboratory, and a nonprofit scientific society. Armed with these examples, workshop participants discussed in breakout and plenary sessions whether there was a need for a laboratory accreditation program and, if a need was perceived, to define what was required. After discussion, workshop participants reached a unanimous decision that there should be a performance-based laboratory testing program and a laboratory accreditation program. The consensus reached was that there should be an **understandable, transparent, trustworthy, performance-based system that accurately depicts whether dreissenids are present in a system.** Workshop participants were also asked to determine the immediate steps towards such a program and to consider how and by whom it should be managed, as well as how it could become sustainable. The conclusions reached included:

(1) A performance testing program should be initiated as soon as possible. Also, a plan for long-term accreditation/certification program should be articulated.

(2) The Round Robin laboratory comparison process should continue to be used as the testing program, and an independent advisory/review committee (with management, technical, and stakeholder representation) should be established to provide oversight.

(3) Efforts should be undertaken in partnership with the Society For Freshwater Science to establish a program to certify the expertise of laboratory staff in the identification of all life stages of Dreissenid mussels.

(4) To sustain the program, various avenues of future funding were brainstormed; however, no specific pathway forward was specified. It was agreed that a detailed budget of anticipated costs should be developed, that stakeholders should be involved, and that diverse efforts would be necessary. These recommendations are available at: www.musselmonitoring.com/Roadmap.

1. Introduction

In 2010, the Quagga-Zebra Mussel Action Plan (QZAP) for Western U.S. waters was developed by the Western Regional Panel on Aquatic Nuisance Species. One of the highest QZAP priorities identified was the expansion of early detection monitoring programs in the Western region. Expansion of early detection programs depends critically on reliable early detection methods and the availability of competent laboratories to complete timely and accurate analysis of plankton samples. With support from the U.S. Fish and Wildlife Service via the QZAP program (FWS-94140-001), two workshops to advance these interdependent requirements were held on the campus of Texas Christian University in Fort Worth, Texas, on February 7-10, 2012.

Workshop 1: "*Dreissena* Early Detection Best Practices," presented on February 7-8, 2-12, focused on identifying the best analytical practices for detection of *Dreissena* spp. larvae. The overall goal of this workshop was to identify the current state-of-the-art methods for the detection and monitoring of *Dreissena* spp. larvae. The intended outcome was that workshop participants should understand the state-of-the-art of cross-polarized light microscopy-based and polymerase chain reaction (PCR) based larval detection assays, that current best practices be recognized, and that future research priorities be identified. To achieve these objectives, the workshop included four components:

- The first component involved three presentations intended to provide a generalized overview of state-of-the art methods for the three most commonly utilized approaches for *Dreissena* spp. larvae detection. Presentations focused on cross-polarized light microscopy (CPLM), image flow cytometry (IFC), and *Dreissena* spp. specific Deoxyribo Nucleic Acid (DNA) detection facilitated by PCR assays. Each presentation was given by recognized experts.

- The second component included two presentations of recent studies where direct comparisons between different *Dreissena* spp. larvae detection technologies could be made.

- The third component involved the presentation of recent technology developments in IFC and PCR.

- The final component involved a series of breakout and plenary sessions with the objective of identifying best methodological practices and immediate research priorities.

Workshop II: "*Dreissena* Early Detection Laboratory Standards," was designed to assess whether there is community-wide support for developing a laboratory

accreditation program to ensure the reliability of *Dreissena* spp. larval detection and, if so, to develop a roadmap for the implementation of such a program. This workshop consisted of two components:

- The first component involved presentations designed to explore the possibilities and ramifications of establishing some sort of laboratory standards program. Presentations were made from three different perspectives:
    - An analytical laboratory currently operating under a rigorous accreditation and standards mandate (drinking water)
    - A commercial *Dreissena* spp. detection laboratory that would be faced with the challenge of meeting new regulatory standards
    - An organization involved in the development and implementation of a standardized training program for taxonomic identification of aquatic species
- The second component consisted largely of breakout and plenary discussions. In breakout sessions, workshop participants were asked to explore and discuss specific questions about the need and options for developing a *Dreissena* spp. early detection laboratory and standards program. In plenary discussions, each breakout group presented a summary of their conclusions and recommendations, which were then further discussed and consolidated into concise statements, which were then voted on by workshop participants.

Appendix I lists the combined participants from both workshops. Over the course of both workshops, a total of 46 individuals representing 26 separate governmental, university, and private agencies participated in the workshops. The first workshop attracted 38 participants from 23 agencies, and the second workshop was attended by 30 participants from 20 different agencies. The majority of participants attended both workshops.

This report summarizes the outcomes from these two workshops. Each report section provides an introduction to the discussion topic, including objectives, participants, and agenda; a synopsis of the presentation, a synopsis of the discussions that took place, and conclusions that were reached.

2. Workshop I: *Dreissena* spp. Early Detection Best Practices

2.1 Introduction (Objectives, Participants, and Agenda)

The goal of the "*Dreissena* Early Detection Best Practices" workshop, held on February 7-8, 2012, was to assess the current state-of-the-art best practices and to develop recommendations for best practice protocols for *Dreissena* spp. presence/absence detection and quantification methods.

Over the course of the 2-day workshop, five expert presentations were given that provided in-depth reviews of existing *Dreissena* spp. larvae detection theory, methods, and recent technology development. In addition, the results of two case studies were presented that allowed for the direct comparison of various *Dreissena* spp. larvae detection.

2.2 Component 1: Early Detection, State-of-the-art Technology – Expert Methods Presentations

This workshop component included three presentations that provided participants with a comprehensive overview of current early detection best practices:

- The first presentation was given by Steven Wells from the Center for Lakes and Reservoirs at Portland State University (PSU). Wells discussed using CPLM and other microscopy-based approaches to detect and identify planktotrophic freshwater mussel larvae in the context of early detection monitoring efforts for zebra and quagga mussels.

- The second presentation was provided by Erin Murchie-Janicki from the National Park Service. Murchie-Janiki discussed the use of imaging flow cytometry instrumentation (FlowCAM) as a means to detect and quantify *Dreissena* spp. larvae in plankton samples.

- The third presentation was delivered by Dr. John Darling from the U.S. Environmental Protection Agency (EPA). It focused on the use of Deoxyribo Nucleic Acid (DNA) based molecular methods for the detection of *Dreissena* spp. larvae in plankton samples.

Following each presentation, there was an opportunity for questions and discussions, with the goal of preparing all participants to actively contribute to the formulation of plans to standardize methods and identify critical future research areas.

2.2.1 Cross Polarized Light Microscopy Presentation – Steven Wells, Portland State University

Synopsis of Presentation. During his presentation, Mr. Wells used, as an example, the procedures used at PSU. The primary goal of his presentation was to highlight technical aspects of the CPLM approach that could be standardized between labs, to communicate his expert opinion on the strengths and weaknesses of the CPLM approach, and, finally, to identify critical research needs.

At PSU, initial collection of larvae involves the use of standard plankton tow nets with a mesh size of 63 micrometers (µm) and preservation in 70-percent (%) ethanol (EtOH). Wells reported that under these conditions, samples remain stable for at least 3 months at room temperature. If lower EtOH concentrations are used, Wells recommended that samples be refrigerated. Wells discouraged the use of isopropyl alcohol, although there was some discussion about this recommendation. Other experts in attendance reported that they have had success using isopropyl alcohol as a preservative. Optimal pH for preservation of veligers is between 6 and 7. Buffering a sample with sodium bicarbonate is possible, but it should be used cautiously because bicarbonate crystals can form and interfere with microscopic analysis. Finally, Wells suggested that, when possible, it is useful to examine samples prior to fixation to observe if larvae are living.

Detection and enumeration of *Dreissena* spp. larvae was the next discussion point. At PSU, prior to microscopic examination, samples are generally prefiltered through a 500-µm mesh to remove larger particles, detritus, and zooplankton. Wells also reported that it can be useful to homogenize plankton samples using a microblender. In his experience, blending does not damage veligers and can free them from sticky materials such as filamentous algae that often occur in eutrophic water bodies. On this point, there was considerable discussion. Veligers can be further concentrated by gravity settling or centrifugation. In Wells' opinion, both methods work. Wells also pointed out that use of density gradient centrifugation to concentrate veligers from a plankton sample is a promising technology, but further research and optimization are required.

Samples can be examined in a variety of types of counting chambers or in Petri dishes. Samples are initially scanned under cross-polarizing light at relatively low magnification (up to 40x) to detect possible veligers based on their shape, size, and birefringence. However, before a larvae can be confirmed to be a Dreissenid, more detailed examination under higher magnification and white light is required. CPLM is considered useful as an initial screening approach. However, taxonomic confirmation requires examination using light or bright-field illumination. Wells reported that, from his own work based on morphological examination,

Dreissena spp. larvae are readily distinguished from other commonly occurring freshwater planktotrophic larvae and ostracods, but Dreissenid species, including the zebra and quagga mussels, cannot be distinguished from each other. The veliger should be photo-documented with high resolution images that include a size scale bar. A low-quality image produced using cross-polarizing light is insufficient.

Finally, Wells discussed decontamination, documentation, and quality assurance/ quality control (QA/QC). Following collection and analysis, all field and laboratory equipment should be thoroughly decontaminated to prevent sample cross contamination. Wells recommended a minimum 4-hour soak in 5% acetic acid or 4% HCl, although bleach (7% solution) is also effective. Throughout the process, rigorous QA/QC procedures should be implemented, including proper training of personnel and detailed documentation of all field collection and analytical activity.

Synopsis of Discussion. There was considerable discussion following Wells' presentation, but there was clearly a general consensus regarding the CPLM procedures that were presented. An important point of discussion following the presentation was the method used for collection. Especially when larval abundance is suspected to be very low, as would be the case during the early stages of an invasion, appropriate sampling was widely believed to be the largest source of error in early detection programs. Bob McMahon, Professor Emeritus at the University of Texas at Arlington) suggested that because veligers are relatively heavy and generally do not swim when first collected, they can be concentrated in live samples immediately following collection by a brief period of settling. There was general interest in this proposal, and it was thought that this approach should be standardized and incorporated into future sampling protocols.

Conclusions. The general conclusions from this presentation are provided below:

- CPLM is effective in identifying freshwater planktrotrophic bivalue larvae in North America. The procedures used by PSU are a good model for a national Standard Operating Procedure (SOP).

- It is difficult to positively identify species levels using only CPLM. Additional methods are needed, such as light or bright-field microscopy at high magnification, and/or molecular methods. Differentiating quagga mussels from zebra mussels is very difficult using only CPLM.

- Standardized documentation and QA/QC procedure are required.

2.2.2 Imaging Flow Cytometry – Erin Murchie-Janicki, National Park Service (Glen Canyon National Recreation Area)

Synopsis of Presentation. In a fashion similar to the previous presentation, Murchie-Janicki used as a template the IFC procedures developed and used by the National Park Service. The goal was to highlight technical aspects of the IFC approach that could be standardized between labs, to communicate her expert opinion on the strengths and weaknesses of the IFC approach, and, finally, to identify critical research needs. All procedures refer exclusively to the IFC instrumentation developed by Fluid Imaging Technology (hereafter referred to as FlowCAM®).

FlowCAM® allows digital images of particles, including *Dreissena* spp. larvae, to be captured from a flowing stream of sample. These images can then be identified and enumerated using dedicated image analysis software. The potential benefits of this approach, compared to CPLM, are increased sample processing speed and elimination of human error. However, it was recognized that these benefits have not yet been fully achieved and that, as of this presentation, the technology is still in development.

Standard procedures at the National Park Service were described. Initially, samples are collected by plankton net tows using a 64-µm mesh size net with a 30-centimeter (cm) opening, essentially as described by Wells in the previous presentation. Generally, the National Park Service targets sample collection areas it believes to be of highest risk including marinas, dams, boat ramps, etc. It also randomly samples other locations within a body of water of interest to provide a larger spatial overview of an infestation and to increase the chance of discovering a new infestation. Samples are preserved in the field with 70% denatured EtOH and, in their experience, can be stored for at least 3 years.

Prior to processing though the FlowCAM® instrument, plankton samples are prefiltered through a series of sieves to remove larger particles. The smallest sieve (top sieve) is 250 µm. A prefiltered plankton sample is necessary to prevent clogging of the instruments' flow cell. It was noted that there could be loss of veligers during this process, but it is necessary to operate the FlowCAM® instrument. Analytical procedures recommended by the manufacturer are followed. However, Murchie-Janicki strongly recommended the use of the 300-µm field of view (FOV) flow cell, compared to the standard flow cell, if quantification is a goal. The instrument is operated at the "Fast" pump setting, and images are captured at a rate of 10 frames per second (FPS). Under these conditions, ~ 0.5 ml min^{-1} of sample can be processed. This volume was less than originally expected, so it still takes a considerable amount of time to process a complete sample. Furthermore, the system requires constant observation because bubbles and clogs can occur in the flow cell. Following each sample, the instrument and flow cell should be thoroughly washed to prevent cross-sample contamination. This also requires technician time.

Once images have been collected, they must be processed. Since images of all particles with birefringent properties are collected, a considerable amount of postprocessing is required. Although automated image analysis based on size and shape using the FlowCAM® software is useful, the National Park Service has found that it is more accurate for a human to review all images, and this can account for a considerable amount of effort associated with operating the FlowCAM®. Furthermore, another problem is that with only images available, it is not possible to further examine a specific particle. This limits the confidence of identification. This is not a problem in samples with high concentrations of *Dreissena* spp. larvae, but it is a problem when they are rare.

Murchie-Janicki concluded her presentation with several best practices recommendations for veliger detection using the FlowCAM® instrument (see table 1, in section 2.5.2).

Synopsis of Discussion. Discussion following Ms. Murchie-Janicki's presentation focused initially on the possible problems with the sample prefiltration requirement. There was concern that larvae could be easily lost during this step. Ms. Murchie-Janicki agreed and reported that their group frequently qualitatively examines materials associated with the prefilters for the presence of *Dreissena* larvae. Some larvae (usually a low number and highly variable) have been associated with this material. Several other technical issues were also discussed including optimal sample flow rates, imaging rates, and automated image analysis. Low flow and imaging rates and unreliable image identification limit the usefulness of the FlowCAM® as a tool to significantly increase the capacity and reliability of *Dreissena* monitoring. Ms. Murchie-Janicki estimated that, on average, they are able to process four to five plankton samples per day using the FlowCAM®, which is not significantly greater than could be analyzed by CPLM in their hands.

Conclusions. IFC is effective for detecting and quantifying *Dreissena* spp. larvae in plankton samples. However, at this point in time, the technology is still developing, and very few laboratories currently have this capability.

2.2.3 Molecular-Based Methods – John Darling, EPA (National Exposure Research Laboratory)

Synopsis of Presentation. Dr. John Darling of the U.S. EPA's National Exposure Research Laboratory provided an overview presentation of a broad range of molecular (DNA) based approaches for detecting, quantifying, and describing the history of aquatic invasive species. In addition to technical issues, Dr. Darling highlighted a myriad of economic and political issues that motivate and drive the development of new technology for the purpose of managing exotic species invasions.

Dr. Darling began his presentation by making the point that there are three primary questions that should be asked when considering the development and usefulness of any molecular method. First, is there a need to determine the presence of a specific species? Second, does a community of organisms need to be described, and third, is quantification required? Answering these questions provides guidance for the choice of an appropriate method. Following this general overview, Dr. Darling provided an informative tutorial that reviewed the theoretical basis for the detection of species and genetic variability based on DNA sequences. He continued with the conclusion that for the purpose of specific species detection, the most common and appropriate method of choice was the use of a PCR assay. This approach utilizes specific primer sets that enable the specific and sensitive detection of *Dreissena* spp. DNA. Application of this method to material derived from standard plankton net tow materials allows the detection of *Dreissena* spp. veliger larvae in the plankton. Use of real time quantitative PCR (qPCR) potentially allows for quantification of larval abundance otherwise not possible with end-point PCR. Theoretically, because of the high abundance of gene targets in a multicellular organism and the exquisite sensitivity of the PCR assay, this approach should yield significant improvement in sensitivity compared to more classical microscopy-based methods. However, there are many technical caveats to this general conclusion. Because Dr. Darling had not worked with *Dreissena,* he did not provide specific information about available *Dreissena*-specific assays, except to defer this discussion to other workshop presentations. Instead, Dr. Darling provided examples of detection of free-DNA, or what is commonly referred to as environmental DNA (eDNA) left by organisms as a unique target for PCR-based exotic species detection methodology.

Dr. Darling continued his presentation by describing several other methodological approaches currently under development that hold great promise for novel molecular-based detection approaches. The methods described included a variety of molecular probing assays including direct probing with oligonucleotide probes, automated sandwich hybridization assays, and novel molecular trapping approaches employing nanotubules. Dr. Darling concluded by making the point that a distinct advantage of molecular based assays, compared to microscopy-based approaches, is that DNA-sequence based methods are readily transferable to other species (not just *Dreissena* spp.), and they can be easily scaled to high throughput analyses that are amenable to automation. These are all fertile avenues for further research. Dr. Darling summarized his main conclusions as:

- There are a number of reasons to think that DNA-based methods will outperform traditional methods for aquatic nuisance species detection. DNA-based methods may also be more easily transferrable to novel monitoring challenges.

- DNA-based methods all use similar principles.

- There are a wide variety of technologies for determining when target DNA has been detected.

- Technology is moving toward increasing automation, decreasing analytical turnaround time, and elimination of PCR amplification steps.

- There are multiple sources of potential error. Some are associated with DNA-based methods themselves, and some are associated with monitoring processes.

- For DNA-based approaches to deliver on their promise, they ultimately will have to stand alone. Therefore, there is a need to develop tools and monitoring processes that managers can trust to provide them with accurate information with known levels of uncertainty.

Synopsis of Discussion. There was generally a great deal of interest in this topic. Considerable discussion focused on the concept of eDNA, particularly for invasive fish species. However, the topic arose regarding whether available *Dreissena*-specific PCR based assays could be better utilized if plankton net tow samples were not the primary target. Water samples that may contain large numbers of *Dreissena* spp. gametes (sperm and eggs) might be a better target. The idea was deemed to be a subject of interest for future research, rather than current application.

Conclusions. There is a great deal of interest, enthusiasm, and optimism concerning the development of molecular-based aquatic nuisance species approaches. It is worthwhile to continue to develop these methods for *Dreissena* spp. specifically and for other invasive species of concern.

2.3 Component 2: Recent Technological Advances – Presentations

Following the presentation of state-of-the-art for CPLM, IFC, and molecular-based methods, two additional short presentations provided recent technology updates. Because of the rapid pace of technology development, especially with respect to IFC and molecular-based methods, presentations focused on these technologies were included.

2.3.1 Imaging Flow Cytometry Updates – Scott O'Meara, Bureau of Reclamation

Synopsis of Presentation. Recognizing the limitations of existing FlowCAM® instrumentation and software for the purpose of *Dreissena* spp. monitoring, beginning in October 2011, the Bureau of Reclamation (Reclamation) and the

developer of FlowCAM® (Fluid Imaging Technology) entered into a Cooperative Research Development Agreement (CRADA) to develop a new FlowCAM® instrument and software for the specific purpose of early *Dreissena* spp. monitoring. This instrument will be called VeligerCAM®.

As Murchie-Janicki discussed previously (see section 2.2.2), several issues have been identified with the existing FlowCAM® instrumentation that negatively impact its ability to be used routinely as a method for early detection and monitoring of *Dreissena* spp. larvae in plankton samples. First, because of slow flow rates, sample processing requires relatively long periods of time; thus, there is not a substantial saving of human technician time. Second, because only CPLM images are captured, there is often not enough information collected to confirm the identity of birefrigent particles that are tentatively identified as *Dreissena* spp. larvae. To address these primary issues, a CRADA was developed to modify FlowCAM® hardware, software, and sample processing capabilities. Instrument modifications included adding a second light source (visible light-emitting device [LED] and infrared light-emitting device [IR LED], adding a second camera and an image beam splitter, replacing the 10-FPS camera with two 30-FPS high-speed cameras, and, finally, increasing the maximum flow rate of the peristaltic flow pump from 0.5 ml min^{-1} to 1.125 ml min^{-1}. Collectively, these advancements significantly improved veliger identification and sample processing speeds.

Experimental testing demonstrated significant improvement with respect to identification and sample processing. However, recovery efficiency of veliger larvae from plankton net tow matrices were well below that achieved by standard CPLM methods. Sample analysis time was also reduced, but it still typically required over 1 hour of technician time per sample. Thus, it was concluded that although significant improvements had been achieved, the instrument was still not optimized for early detection of larvae. Work is continuing to improve the VeligerCAM®.

Synopsis of Discussion. A short discussion followed Mr. O'Meara's presentation. Victoria Kurtz from Fluid Imaging Technology was in the audience and also fielded questions. As was pointed out during the presentation, it was recognized by the participants that the VeligerCAM® is not ready to be routinely used as a method to improve early detection of *Dreissena* spp. However, the workshop participants were quite enthusiastic concerning the considerable improvements that have been made and highly encouraging of continued efforts to improve the instrumentation. Ms. Kurtz was asked if the instrument was available for purchase and, if not, when it would be available. Ms. Kurtz said she did not know, but she thought it would be at least a couple more years. However, she did offer that cross polarizing filters are available for current models of the FlowCAM® that allow routine monitoring of *Dreissena* spp. larvae in plankton samples.

Conclusions. Improvements of the FlowCAM® technology are encouraging, and the community is supportive of its continued development.

Post Workshop Update. Since the workshop, researchers at Reclamation and Fluid Imaging Technology have continued to optimize the VeligerCAM® instrument and operating protocols. Denise Hosler, the lead investigator of the project at Reclamation, reported that as of November 2013 Reclamation was using the VeligerCAM® routinely with plankton samples with high veliger counts and that it has reduced sample processing time and improved count accuracy and reliability. Reclamation will publish these results in an upcoming Technical Report.

2.3.2 PCR Updates – John Wood, Pisces Molecular LLC

Synopsis of Presentation. In view of the diversity of available PCR methods that exist (many of which are still in development) and a current limitation of PCR-based approaches to quantify the abundance of *Dreissena* spp. larvae in a given sample, Dr. Wood provided a technical update of work that his company, Pisces Molecular LLC, is currently conducting to address some of these issues.

Beginning from the premise that the realization of standardized *Dreissena* spp. PCR-based detection and quantification assays will eventually require quantitative comparison between laboratories and methods, Dr. Wood proposed that a solution may be to develop a universal PCR standard that could be used to cross compare between labs and methods and that would allow the establishment of absolute quality and sensitivity benchmarks. To achieve this goal, Dr. Wood proposes the development of a recombinant plasmid containing all of the various genes (markers) that are currently used in *Dreissena* spp. detection PCR-based methods. Pisces Molecular LLC is working on the development of such a plasmid and expects it to be available commercially by 2013. (As of June 2013, the plasmid is constructed and awaiting dilution and exact quantification; it should be ready by the end of the summer.)

An important question concerning the utility of PCR-based methods is whether they can be used to provide quantitative estimates of larval abundance. Deriving biologically meaningful estimates of larval abundance (i.e., larvae per unit volume) is not at all straightforward based on PCR methods. Quantitative PCR (qPCR) assays provide estimates of the number of target gene copies per sample, not the number of total larvae. In the case of *Dreissena* spp. larvae, the number of any given gene target per larvae is not known and could be variable. Dr. Wood, in his presentation, provided the results of a small study in which the number of PCR gene targets was quantified using qPCR in approximately 50 *Dreissena* spp. larvae collected from the St. Laurence River during a single growing season. Remarkably, the number of target genes per larvae varied by over 4 orders of magnitude from 100 to 1 million copies per larvae. Furthermore, there was not an

obvious correlation between gene copy number and the size of larvae (size as a rough proxy for age). These results suggest that qPCR may never be able to provide absolute quantification of the number of larvae per sample.

Synopsis of Discussion. There was considerable technical discussion concerning the development of a universal PCR standard, but there was consensus that such a tool would be useful. However, many participants voiced concern about the sole reliance of method standardization and quality control based only on the detection of plasmid DNA. Most of them agreed that the availability of reference whole veligers was also required and could not be completely replaced by the availability of a reference purified DNA standard.

The recognition of gene copy abundance variability in *Dreissena* spp. larvae was acknowledged to have significant implications concerning the absolute quantification of *Dreissena* spp. larvae by PCR-based methods.

Conclusions. Development of a universal PCR standard is underway and may be useful for future development of standardized *Dreissena*-specific PCR protocols. However, most participants agreed that whole veligers were required for reference materials and could not be replaced by a plasmid standard. Absolute quantification of larval abundance may not be possible by PCR-based approaches due to the high variability of PCR gene target copy number (specific genes) present in individual larvae. Continued research is required to address this important issue.

2.4 Component 3: Methods Versus Results – Quantitative Comparisons Between Microscopy and PCR Results from Real-World Case Studies – Presentations

In the recent past, there have been a number of examples in which conflicting results concerning the presence of *Dreissena* spp. larvae were obtained using microscopy-based and molecular-based detection methodologies. A major concern of the management community is improving the understanding of these discrepancies and developing independent and self-consistent *Dreissena* spp. larvae early detection methodologies. Therefore, as a component of the Best Practices Workshop, the results from two recent real-world case studies were presented. Results from monitoring efforts in Lake Texoma and a community-wide, double blind, round robin study were presented. In both of these studies, the same samples had been evaluated with different methods, so the results could be directly compared. Dr. Marc Frischer from the Skidaway Institute of Oceanography and Christopher Churchill of the U.S. Geological Survey (USGS) presented the results from these studies.

2.4.1 Community-Wide, Double Blind Methods, Round Robin Study – Marc Frischer, Skidaway Institute of Oceanography

Synopsis of Presentation. Dr. Frischer presented the results of a double blind, round robin methods experiment that examined the reliability and accuracy of CPLM, IFC, and PCR-based *Dreissena* spp. veliger detection in standardized reference plankton samples.

This study was conducted and completed in 2010 and involved the participation of 19 different laboratories that were actively performing plankton sample analyses for *Dreissena* spp. detection at the time the study was conducted. The basic study design involved reference *Dreissena* spp. spiked plankton samples which were sent to each laboratory for analysis. Following completion, each lab submitted their results and a detailed description of the methods that were used. Overall, CPLM proved to be the most reliable method for both detection and quantification. IFC was the second most reliable method, but it tended to quantitatively underestimate larval abundance. PCR was the least reliable method for detection, and quantification was not reported. The most common errors were false negatives, indicating that all methods were more likely to miss a detection rather than falsely report the presence of *Dreissena* spp. larvae in a plankton sample.

Comparative analysis of the specific methodology used did not reveal any clear explanation for methodological discrepancies. However, all of the highest performing laboratories were considered the most experienced (with at least 3 years of specific experience) analyzing plankton samples for the presence of *Dreissena* spp. larvae.

Frischer concluded the presentation with eight specific recommendations:

1. At the present time, CPLM analysis should be used as the primary approach for early detection of *Dreissena* spp. larvae. All potential detections should be documented with high quality photomicrographs.

2. Develop a comprehensive CPLM training program and expand current microscopy capacity and expertise.

3. Establish a group of certified experts who are capable of reliably identifying *Dreissena* spp. larvae from good quality micrographs.

4. Fund research to improve accuracy of IFC as a routine method for detecting and enumerating *Dreissena* spp. larvae, especially when larvae are at very low concentrations.

5. In association with laboratories currently utilizing IFC, they also routinely conduct CPLM analysis to facilitate cross-comparison between these methods.

6. Fund research to identify the causes of variability in PCR methods. Conduct a methods "cook-off" study involving the most experienced PCR laboratories to evaluate all currently available methods.

7. Conduct technical workshops to discuss the results of this study and a broader review of PCR diagnostic methods. The product of such workshops should be the development of specific research recommendations.

8. Establish a laboratory certification standard for all types of detection methods for *Dreissena* spp. larvae.

The results of this study have been published in the journal *Lakes and Reservoir Management* (Frischer et al., 2012).[1]

Synopsis of Discussion. There was considerable discussion regarding the actual results and implications of this study. It was recognized that each method was capable of near perfect reliability; however, each method was also subject to different sources of errors. Full discussion of the recommendations was deferred to breakout and plenary discussions. It was generally concluded that this type of study is useful and required to gauge the continued efficacy of methodological improvement and to assist management in interpreting real-world field results. It was believed that a much larger study should be conducted in the near future as methods continue to improve. A new study should contain a larger number of reference samples (perhaps only samples with low numbers of larvae). For the PCR-based assays, the inclusion of the universal plasmid standard Pisces Molecular LLC is developing (see section 2.3.2) should be incorporated.

Conclusions. Each of the examined methodological approaches has the potential for accurate and reliable detection of *Dreissena* spp. larvae in plankton samples. However, as of 2010, CPLM remains the most reliable method. Continued efforts to develop IFC and PCR-based methods are warranted and necessary.

[1] Frischer, M.E., Kevin L. Kelly, and Sandra A. Nierzwicki-Bauer. 2012. "Accuracy and reliability of *Dreissena* spp. larvae detection by cross-polarized light microscopy, imaging flow cytometry, and polymerase chain reaction assays," *Lake and Reservoir Management,* 28: 265-276.

2.4.2 Lake Texoma, Texas: A Case Study – Christopher Churchill, U.S. Geological Survey

Synopsis of Presentation. Mr. Churchill presented the results from 2 years' worth of *Dreissena* spp. monitoring in Lake Texoma in north Texas. The presence of zebra/quagga mussels in Lake Texoma is of great concern because Lake Texoma is an important water reservoir for north Texas and because of its connectivity (through pipes) with other watersheds. Therefore, it is a likely gateway for the continued expansion of *Dreissena* spp. mussels in the Southwestern U.S.

The ongoing monitoring program in Lake Texoma involves regularly (weekly) sampling the lake and examining samples using CPLM. Representative samples are also analyzed by PCR at Reclamation. Thus, the data set provides a good opportunity to compare the results from these two methodological approaches.

Two years' worth of monitoring data (2010 and 2011) were presented. In both years, veligers were detected throughout the year; however, veliger abundance varied significantly over this period and between sampling sites. Veliger concentrations ranged from 0.01 - > 2 veligers^{L-1}. There was a 97.5% agreement between the microscopy and PCR results. In the case where there was a discrepancy between the two methods, *Dreissena* spp. were detected by PCR but not by CPLM. This event occurred in a location called Sister Grove Creek after a pipe connection to Lake Texoma had been closed. At the time of detection, adult quagga mussels were present at the site. It was postulated that the detection could have been due to the presence of *Dreissena* spp. eDNA, rather than veligers, but this hypothesis was not rigorously explored.

Synopsis of Discussion. Many of the sampling and analytical details of the study were further discussed and clarified. It was pointed out by Kevin Kelly from Reclamation that, in his opinion, such close agreement between CPLM and PCR results was due to the decision to examine split samples, rather than process samples for PCR after they had been processed for CPLM. Several other workshop participants, including Mr. Churchill, agreed with Dr. Kelly's comment. The case involving positive PCR detection, but negative CPLM detection, in Sister Grove Creek was discussed further, and the likelihood of eDNA detection versus the possibility that larvae were present was considered. However, as Mr. Churchill concluded during the presentation, there is not enough data available to reach a definitive conclusion.

Following the discussion of technical issues, there was an extended discussion concerning the response of the management and stakeholder (water utility) communities to the presence of *Dreissena* spp. in Lake Texoma and the response/use of the monitoring data provided by the USGS. Mr. Churchill reported that a very healthy and honest dialog existed between all groups. He believed that all of the partners share the objective of doing everything possible to

limit the spread of *Dreissena* spp. mussels in north Texas. All of the workshop participants were impressed by this relationship and reached the conclusion that Lake Texoma represents a good model for the development of management partnerships.

Conclusions. The science based monitoring and management plan appears to be effective in limiting the spread of *Dreissena* spp. mussels from Lake Texoma. Since pipe connections between the Lake and adjacent water bodies have been closed, intense monitoring efforts indicate that mussels have not yet spread out of the lake. In the case of Lake Texoma, monitoring results based on both CPLM and PCR analyses appear to be in close agreement, and the management strategies appear effective in slowing the spread of *Dreissena* spp. mussels.

2.5 Component 4: Recommendations for Best Practices and Future Research and Management Priorities – Breakout and Plenary Discussion

Interspersed throughout the workshop, a series of breakout (small group) discussions were conducted. The first goal of the breakout discussions was to develop recommendations for best practice protocols to detect and quantify the presence or absence of *Dreissena* spp. veligers using CPLM, IFC, and PCR and then come to a consensus. Breakout discussion groups were organized on the basis of methodology, with participants self-selecting the group they wished to participate in. Each breakout group was assigned a discussion leader and official note taker. Following these breakout discussions, each group was responsible for presenting the recommendation of the group in plenary discussion and leading a conversation that resulted in a series of recommendations.

A second goal of the best practices workshop was to review the recommendations for *Dreissena* spp. early detection and research priorities derived from the round robin II study presented earlier by Frischer (see section 2.4.1) and to reach a consensus on the most important steps for continued improvement and current use of available early detection methodology. Recommendations were voted on by all participants using a "sticky dot" consensus building strategy called "Dotmocracy." Each participant was given 3 votes, and the number of recommendations considered was limited to 10 or less.

2.5.1 Best Practices – CPLM

David Britton led this group discussion. As a group, each component of the existing protocol presented by Wells (see section 2.2.1) was systematically discussed. Specific methodological components included sampling, sample preservation, microscopy technique, QA/QC procedures, documentation, and certification of results.

Sampling. A recommendation was made to use standard plankton nets with a mesh size of approximately 63 μm. Both vertical and horizontal tows are appropriate, depending on the actual sample site. Sites deeper than1 meter should be sampled vertically. Care should be taken to avoid sediment collection.

Sample Preservation and Storage. There was considerable discussion on this subject. Ethanol was agreed upon as the best preservative; however, there was less consensus on the most appropriate concentration. Most participants recommended that 25% ethanol should be used and that multiple samples (at least two) should be collected. In addition, most participants agreed that storing ethanol preserved samples at room temperature was sufficient but that keeping them cold (4 degrees Celsius [ºC]) would not damage them. Keeping the samples in the dark was also recommended. Samples appear to preserve well, in most cases, for at least several months; however, it was agreed that samples should be analyzed as soon after collection as possible.

CPLM. Samples should be initially screened under cross-polarizing light at low magnification. Birefringent particles should be examined further under white or bright-field illumination at higher magnification and confirmed as a *Dreissena* spp. veliger based on morphological characteristics including size, shape, and presence of a velum. High quality images should be taken of representative veligers detected. These images can be shared with experts for confirmation. It is not sufficient to capture only images under cross-polarizing illumination. Photomicrographs captured using white or bright field illumination are also required.

QA/QC. A detailed protocol and checklist should be established and followed. Each sample collected should be uniquely identified. Sample chain of custody documentation should be completed. All technicians should receive training, and training records should be maintained.

During the plenary discussion, the development of standardized protocols was recommended and could be based on the protocols used at PSU by Steven Wells and Mark Systema.

It was recognized that an authority should be identified to implement this final recommendation; however, the identification of this authority was considered beyond the scope of the workshop.

2.5.2 Best Practices – IFC

Erin Murchie-Janicki led this group discussion. During her state-of-the-art presentation (see section 2.2.2), she provided a template for discussion of best practice protocols, which was used to guide breakout group discussions.

Discussion was broken into eight categories including: (1) experimental (sample collection) design, (2) sample collection, (3) sample handling and storage, (4) sample preparation for IFC analysis, (5) sample analysis, (6) QA/QC, (7) equipment, and (8) statistical analysis and result reporting. A summary of these discussions and conclusions is provided in table 1.

In plenary discussion, and as presented earlier in the state-of-the-art and IFC technology update presentations (see sections 2.2.2 and 2.3.1), it was recognized that IFC technology for early detection and quantification of *Dreissena* spp. larvae in plankton samples is still in active development. Therefore, it was recognized that these recommendations should be viewed as guidelines, rather than standard protocols.

Table 1. Best Technical Practices for Veliger Detection Using FlowCAM – Standard Protocol Recommendations

Category	Recommendations
1) Sample Collection Design a. Site selection b. Frequency	• Refer to 2009 Reclamation Plankton Sampling Protocols (1.a., b.) or other published protocols; should align with protocols for CPLM and PCR. • **Site:** Targeted "higher risk" routine locations (marinas, launch ramps, inflows, and random sampling locations) and where visitors enter the lake. Also, spot check random locations. • **Frequency:** Dependent on water body size, usage; "routine (targeted areas)" more than twice monthly, and "random" locations monthly. • Sample during spawning (> 9 °C). • With limited funds, take more samples during peak spawning temperatures and less samples when it is very cold or very hot (temperatures estimated, but not rigidly set, at 16-19 ºC). • For early detection, it is best to collect numerous samples.
2) Sample Collection a. Plankton tows/net/depth b. Dealing with sediment c. Net rinsing/ decontamination d. Other data collected	• Refer to 2009 Reclamation Plankton Sampling Protocols (1.c., 2.a., 2.b.), SM 10200 B (22nd edition), and other published protocols; should align with sample collection protocols with CPLM and PCR. • 63/64-µm pore size, simple conical net, weighted cod-end, 30-centimeter (cm) net opening. • Vertical plankton tows (up to 50 meters [m]), as close to the bottom as possible without disturbing the bottom, or no deeper than the thermocline (dependent on your body of water). • Horizontal plankton tows are better than nothing if you are in a very shallow body of water.

Table 1. Best Technical Practices for Veliger Detection Using FlowCAM – Standard Protocol Recommendations

Category	Recommendations
	• Consider pumping technique for sample collection. • Discard and recollect sample if sediment is present in cod-end. • Keep the net submerged for about 30 seconds and then retrieve it at about 0.5 meter per second (m/s). • Rinse the sample down into the cod-end with lake water (do not submerge the top ring); repeat. • Transfer to the sample bottle, then rinse remnants in cod-end into the sample bottle. • Decontaminate plankton net and cod-end in 5% acetic acid (white vinegar). Note: If you are also using your sample for PCR, this procedure is not recommended because it exposes DNA cross-contamination to your sample. Having a new net for each body of water sampled is recommended. ○ Spray, soak (for how long?): Further studies are needed to make determinations on decontamination processing. • **Collect/record on data sheet:** Try to record the following information seasonally for each water body (not necessary for each sample): global positioning system (GPS), water temperature, depth to bottom, depth of tow, date, time, site identification and description, preservation and decontamination, field notes, who collected the sample, pH, hardness, temperature, calcium levels, conductivity, dissolved oxygen, turbidity, phosphates, nitrogen, chlorophyll.
3) Sample Handling and Storage a. Sample bottles b. Preservation c. Labeling d. Chain of custody	• Sealed polypropylene in 125-500-milliliter (mL) bottles. • Preserve in 20-70% dependent on needs EtOH, store up to 3 months before analysis. • Buffer sample recommended. • Label with site identification, date, depth, and preservation. • Build chain of custody into field and laboratory forms.
4) Sample Preparation for FlowCAM Analysis	• When using 300-µm flow cell FOV, filter out large particles using 210-µm top sieve (subject to modification), 63-µm bottom sieve (useful to concentrate but risks sample loss). If no bottom sieve, initially filtering directly into a beaker will prevent sample loss but increase volume and process time. • Keep larger sized sample; back rinse filters to best remove sample. • Analyze sample in 63-µm sieve or effluent in beaker in FlowCAM.

Table 1. Best Technical Practices for Veliger Detection Using FlowCAM – Standard Protocol Recommendations

Category	Recommendations
	• Add surfactant to sample to be analyzed in FlowCAM – dilution is not exact (several drops).
5) Sample Analysis Using FlowCAM a. FlowCAM settings b. FlowCAM setup c. Running the sample d. Postprocessing e. Postprocessing filters	• **Settings:** 4x microscope objective, polarizing filters, 300-µm FOV flow cell is preferred (can use standard as well), intensity mean about 25, auto-image mode. • Load context file with settings appropriate for veliger detection (this is preloaded by Fluid Imaging Technologies). • Pump is run on "Forward Fast – 5", 10 FPS for peristaltic pump. • If using a syringe pump, context setting should reflect a 30-FPS analysis.
	• **Setup:** Prime with DI water (manual prime setting on pump). Ensure that no air is in the flow cell, let the DI water travel through the sample pipette tip (sample feeder) past the silicone tubing pipette junction, and then introduce a drop of sample to use for focusing objective on plankton sample in flow cell (raise intensity mean to about100). Once focused, reverse pump to return all sample back to pipette tip prior to starting the run, and then return intensity mean to 25. • **Running sample:** Background is automatically calibrated upon start of run; add small amounts of sample to prevent clogging, dilute if necessary. • The run can take up to 1 hour, depending on volume. • When all of the sample has gone to its effluent, introduce DI water to pipette, and then run completely through the tubing until dry. Turn off pump, detach tubing, and force DI water with squirt bottle tip through end of silicone tubing to ensure that any remaining sample in tubing has been returned to the analyzed sample. • Decontaminate tubing and flow cell with white vinegar for a minimum of 1 hour. • **Postprocessing:** View collage images and select particles of interest (*Corbicula*, ostracods, veligers, unknown). • Export data for selected images and find source images for each particle of interest. • Review source image (export and measure if necessary) and identify organisms. Record the number of *Corbicula*, ostracods, veligers, and unknowns, if any.

Table 1. Best Technical Practices for Veliger Detection Using FlowCAM – Standard Protocol Recommendations

Category	Recommendations
	• If unknowns exist, send images for second opinion. • If possible, analyze samples with CPLM. • Save all collage images, as well as all source images of particles of interest. • **Using filters:** Filters may be used in postprocessing to decrease the number of collage images to be reviewed. Although a filter is a powerful tool, if used incorrectly, it may end up excluding some images that might be of interest. Filters are not recommended for postprocessing unless they have been extensively tested and proven to not exclude potential particles of interest. Suggestion: Have a large library developed and made available to laboratories with FlowCAM to enable preliminary detection of veligers in the image.
6) QA/QC a. Prevent cross-contamination b. Sample loss c. Maintaining constant conditions	• **Prevent cross-contamination:** Designate a net for each water body. • Decontaminate all equipment (field and laboratory) using 5% acetic acid or white vinegar (for how long?) between sample sites and sample analyses (the team questions the effectiveness of vinegar for decontamination). • Rinse equipment with DI or tap water (lake water is acceptable in some circumstances). • **Prevent sample loss:** Rinse with DI water all field and laboratory equipment that comes into contact with the sample, or that is used to store or transfer the sample (refer to 2009 Reclamation protocols). • **Maintaining constant conditions:** Detailed SOPs and quality manual, training documentation and demonstration of capability for technicians, equipment documentation for FlowCAM, chain-of custody procedures (built into field and laboratory datasheets?) • **Sample storage:** Store the sample in ethanol to preserve it (depending on % of ethanol, refrigerate sample if possible). It is recommended that a small amount (1 teaspoon) of baking soda be added if the pH is less than 6.9. Refer to 2009 Reclamation protocols. • **Database backup:** External hard drive or network server.
7) Equipment: a. Field b. Laboratory c. Preservative and	• **Field:** Boat, simple conical plankton net with 63/64-µm pore size mesh, 30-cm opening, weighted cod-end (removable), metered line (50 m) and reel, polypropylene sample bottles (125-250 mL) with sealing lids, field sheets, permanent marker,

Table 1. Best Technical Practices for Veliger Detection Using FlowCAM – Standard Protocol Recommendations

Category	Recommendations
decontamination d. For FlowCAM	pen, GPS unit, field thermometer. • **Laboratory:** If splitting sample, Folsom plankton splitter; 63-µm bottom sieve (if using), and 210-µm top sieve (subject to modification) for filtering; small beaker, funnel, disposable pipette, pipette tip. • **Preserve and decontaminate (for both field and lab work):** Absolute EtOH, 5% acetic acid or white vinegar, DI water, spray and/or wash bottles. • **For FlowCAM analysis:** FlowCAM, 300-µm flow cell (prefer FOV), silicone tubing, 4x objective, polarizing filters, Visual Spreadsheet, and proper context settings/file (acquire directly from Fluid Imaging Technologies).
8) Statistics and Data Reporting	• Visual Spreadsheet produces reports that include basic statistics and data on imaged particles. • Database should include site name, storage conditions, water chemistry parameters, sample date, depth to bottom, tow depth, surface water temperature, GPS coordinates and datum, type of flow cell and objective used, and count data for *Corbicula*, ostracods, veligers, and unknowns. • Collage images and source images for particles of interest are saved. • Annually, database is distributed to program managers and partner agencies. Reports summarizing sampling efforts and FlowCAM analysis results should be completed annually and accompany the database.

2.5.3 Best Practices – PCR

John Darling, from EPA, led this group discussion. To guide it, Dr. Darling provided a draft set of recommendations (table 2). Discussion was broken into six categories: (1) sample collection, (2) sample preparation, (3) assay specificity, (4) assay sensitivity, (5) quality control, and (6) statistics and reporting. During these discussions, it became clear that not enough data existed to decide the best practices for DNA-based (PCR) detection of *Dreissena* spp. larvae in plankton samples.

In plenary discussion, and as presented earlier in the state-of-the-art and DNA-based detection technology update presentations (see sections 2.2.3 and 2.3.2), PCR technology for early detection and quantification of *Dreissena* spp.

larvae in plankton samples was recognized as still in active development. Therefore, the technical experts and workshop participants believed it would be premature to develop a best practices standard PCR-based protocol for this technology at its current stage of development.

Following presentation and discussion of each method, it was proposed that establishing assay detection sensitivity benchmarks that could be applied equivalently to all early detection assays might be more useful than establishing standard protocols for each of the detection approaches.

It was concluded that all *Dreissena* spp. early detection SOPs should be capable of reliably detecting *Dreissena* spp. larvae in plankton samples at

concentrations of 10- 50 m^{-3} (0.01- 0.05 $^{L-1}$). This recommendation was approved by a unanimous vote of the participants who identified themselves as technical experts.

Table 2. Partial Draft Recommendations for Best Practices for DNA-Based Detection Methods

1.	Sample collection
a.	Refer to SOPs for plankton sampling protocols (Reclamation or other); adhere to protocols that limit sediment collection; adhere to all QA/QC protocols to eliminate cross-contamination.
b.	Target high risk locations for routine sampling (marinas, launch ramps, etc.), as well as random sampling, depending on available level of effort (can we use modeling efforts—larval biology, hydrology, etc.—to predict areas with the highest likelihood of detection?)
c.	Sample known uninvaded sites (if possible) to ensure assay specificity.
d.	Sampling frequency will depend on available level of effort and estimated risk.
e.	Resample all sites with positive detections as soon as possible after detections are reported.
2.	Sample preparation.
a.	Preservation for DNA-based tests may be incompatible with preservation for morphological tests.
b.	Need to empirically determine the most appropriate preservation technique (is 70% ethanol sufficient?)
c.	Minimize storage of samples prior to analysis.
d.	Standardization of DNA extraction—what method will work for all samples (including those with large excess of nontarget biomass, those with sediment, etc.)?
3.	Assay specificity.
a.	Conduct in silico analysis for all probes/primers; determine mismatches against both targets and nontargets; conduct short oligo BLAST to assess potential recognition of unintended targets.
b.	For in vitro analysis of PCR approaches, conduct random sequencing of products to confirm specificity; for deployment, conduct sequence analysis for all positive detections.
c.	Test specificity (in silico and/or in vitro) against all known invasive or potentially invasive variants of target species (assess broad geographic genetic variation).
d.	Test specificity against, at a minimum, all nontarget congeners and all nontargets from the same family known to occur in sampled waters; test probes against samples from known uninvaded sites with community composition similar to those being tested.

Table 2. Partial Draft Recommendations for Best Practices for DNA-Based Detection Methods

4.	Assay sensitivity.
a.	Test and report "process" sensitivity limits (e.g., number of propagules per unit sample volume, determined by spiking known propagule numbers in realistic sample volumes) (i.e., the volume of a typical plankton tow).
b.	Assess impacts of nontarget biomass on sensitivity by testing for detections in samples with known quantities of nontarget biomass.
c.	Assess impacts of environmental contaminants (e.g., PCR inhibitors) on sensitivity (e.g., by testing for inhibitory effects of filtered sample water).
5.	Quality control.
a.	Implement appropriate controls at all process stages using blanks and spiked samples.
i.	Sample collection (dummy sample control).
ii.	Sample processing (e.g., filter controls).
iii.	DNA extractions.
iv.	Method control (e.g., PCR amplification control).
b.	Develop SOP for sample chain of custody.
c.	Define standards for "positive" detections.
i.	For qualitative detections (e.g., standard PCR), use image densitometry of gels and cutoff thresholds or, at a minimum, require multiple blind observer reports on detections.
ii.	For quantitative detections, determine cutoff thresholds for positives, based on known detection limits.
d.	Ensure repeatability (i.e., multiple detection tests run on the same processed sample by the same researcher).
e.	Ensure reproducibility (i.e., agreement between results of assays conducted on the same sample by different researchers at different times (ideally in different laboratories).
f.	Adopt common SOPs for laboratory notebooks, molecular approaches (e.g., PCR workflow), etc.
6.	Reporting and statistics.
a.	Determine false negative and false positive rates for molecular assay—how many tests need to be done for such reports to be useful?
b.	Report positives based on standard definitions (see above).
c.	Report spatial and temporal distribution of positive reports to assess patterns of detection.
d.	Report variables associated with collected samples (GPS coordinates, tow depth and volume, water temperature, overall biomass, collection equipment used, and collector identification, etc.).

2.5.4 Recommendation for Early Detection Best Practices and Future Research Priorities

The final plenary item of the Best Practices Workshop was to vote on the recommendations developed based on the Early Detection Round Robin II study. These recommendations had been discussed earlier in the workshop and reviewed during each of the breakout sections. The results of the voting are listed below.

Ranked priorities of Round Robin II study recommendations. Only recommendations that garnered votes are listed. Each of the original recommendations from the RRII report is provided in section 2.4.1 and available at: www.musselmonitoring.com/Report%20Recommendations.pdf

1. Fund research to identify the causes of variability in PCR methods. Conduct a methods "cook-off" study involving the most experienced PCR laboratories to evaluate all currently available methods. Upon completion of the study, conduct a technical workshop to discuss the results and undertake a broader review of PCR diagnostic methods. The product of this workshop should be the development of specific research recommendations. [26 votes]

2. Establish a laboratory certification standard for all types of detection methods for *Dreissena* spp. larvae. [16 votes]

3. CPLM analysis should be used as the primary approach for early detection of *Dreissena* spp. larvae. All potential detections should be documented with high quality photomicrographs. [16 votes]

4. Establish a group of certified experts who are capable of reliably identifying *Dreissena* spp. larvae from good quality micrographs. **[11 votes]**

5. Develop a comprehensive CPLM training program and expand current microscopy capacity and expertise. **[8 votes]**

6. Fund research to improve accuracy of IFC as a routine method for detecting and enumerating *Dreissena* spp. larvae, especially when larvae are at very low concentrations. **[1 vote]**

The workshop was adjourned at 4 p.m. on Wednesday, February 8, 2012.

3. Workshop II: *Dreissena* Early Detection Laboratory Standards

3.1 Introduction (Objectives, Participants, and Agenda)

The first goal of the "*Dreissena* Early Detection Laboratory Standards" workshop, held on February 9, 2012, was to determine if there was a consensus regarding the need for a laboratory accreditation program and, if so, what the scope should be for such a program. If a need and scope were determined, the second goal of the workshop was to outline a roadmap for developing and implementing such a program.

Appendix I identifies the combined participants from both workshops. The workshop attracted 30 participants from 20 different agencies.

Over the course of the 1.5 day workshop, four presentations were given. The purposes of the presentations included: (1) familiarizing participants with the current recognized need for a laboratory accreditation program, (2) providing an example of existing programs, (3) sharing a variety of perspectives from potential participants on developing and implementing a quality assurance and accreditation program for *Dreissena* early detection and monitoring, and, (4) creating a proposal for developing a *Dreissena* spp. early detection (microscopy-based) certification program. In parallel with these presentations, the workshop included a number of small group and plenary discussion sections with the explicit goal of synthesizing recommendations and developing an outline for advancing a *Dreissena* spp. laboratory standards program. The workshop agenda is provided as appendix III.

3.2 Component 1: Expert Presentations

Over the course of the workshop, presenters included Stephen Phillips, from the Pacific States Marine Fisheries Commission; Dr. Ellen Braun-Howland, Director of the drinking water safety program at the New York State Department of Health Environmental Biology Laboratories; Dr. John Wood, the President of Pisces Molecular LLC, a commercial diagnostics company; and Dr. John Morse, the co-chair of the Taxonomy Certification Program operated by the Society for Freshwater Sciences.

3.2.1 Needs for Laboratory Accreditation (Certification Program) for *Dreissena* Monitoring – Stephen Phillips, Pacific States Marine Fisheries Commission

Mr. Stephen Phillips, from the Pacific States Marine Fisheries Commission, was asked to provide an update to workshop participants concerning the efforts of the Western Regional Panel Dreissenid Detection Standards and Protocol Coordination Working Group (WRP) and the Invasive Species Advisory Committee (ISAC) of the National Invasive Species Council. Mr. Phillips led the WRP and, when it was integrated into the ISAC, has continued as a member. These groups are charged with the development and implementation of invasive species management in the U.S. and were established for this purpose during the Clinton administration. During his presentation, Mr. Phillips provided a management perspective concerning the need for a laboratory accreditation process and the results of a survey of *Dreissena* spp. detection laboratories as a result of the ISAC's work on *Dreissena* spp.

Mr. Phillips began by stressing that, from the perspective of an invasive species manager, it is essential that reliable and accurate detection and monitoring data be available. In the case of *Dreissena* spp., early detection data has often been inconclusive or appears to be unreliable, consequently making it difficult to create and defend management policy and action. Currently, most management plans require at least two independent and verified detection events before action plans can be implemented. Due to the difficulty in obtaining reliable *Dreissena* spp. early detection and monitoring information, in 2010, the WRP conducted a survey of laboratories involved in the analysis of plankton samples for the presence of *Dreissena* spp. larvae. Most of the laboratories surveyed had participated in the double blind methods comparison study, described by Dr. Frischer earlier in this workshop (section 2.4.1). A total of 28 laboratories were invited to participate and 18 responded. In general, the majority of laboratories shared the view of the management community and perceived a need for a laboratory accreditation program. Most labs also indicated a willingness to participate in an accreditation program if costs were relatively low and only essential performance information was made publically available.

Following the completion of this study, the WRP was merged into the ISAC group, who commissioned the development of a white paper to define the state of the knowledge of PCR-based techniques for the detection of exotic invasive species. The motivation for developing this paper was that regulatory officials and industry representatives had expressed the need to ascertain the validity of an assay's performance in terms of sensitivity, specificity, accuracy, and robustness, as well as to establish an accreditation program to benchmark laboratory performance. The ISAC group came to the conclusions that: (1) confidence in the effectiveness of DNA-based tools and the laboratories that implement them will be best ensured through rigorous examination of assay design and independent assessment of quality control measures, (2) sampling strategies must

be designed in a way that inferences regarding the presence of aquatic invasive species can be rendered robust to the potential for errors, and (3) future research should aim to better understand the relationships between DNA-based detections and the presence of target organisms. Furthermore, ISAC recognized that DNA-based monitoring approaches, including the use of eDNA, have the potential to outperform conventional monitoring approaches, but only if carefully designed studies continue to validate this approach and provide a comprehensive and standardized protocol. An immediate need was recognized for methodological developments to provide invasion biologists with standardized and user-friendly protocols.

Mr. Phillips concluded his presentation by summarizing his perspectives on efforts to improve invasive species management in the United States; in particular, the ongoing *Dreissena* spp. invasion in the Western U.S. Mr. Phillips suggested that, ideally, a comprehensive monitoring program that included the use of PCR (DNA)-based methods would be established under one the of member federal agencies of the National Invasive Species Council. Currently, at least two federal agencies have some level of regulatory control regarding PCR assays developed and validated for marketing in the United States. The Federal Drug Administration (FDA) is responsible for enforcement of the Federal Food, Drug, and Cosmetic Act that covers *in vitro* diagnostic devices which are a subset of medical devices "intended for use in the diagnosis of disease and other conditions, including determination of the state of health, to cure, mitigate, treat, or prevent disease or its sequel." The Animal and Plant Health Inspection Service through the Center for Veterinary Biologics regulates the licensing and sale of diagnostic kits used in detecting animal diseases under the authority of the Virus Serum Toxin Act. Both agencies are involved in assuring that commercially available kits for running assays are safe, effective, reliable, and truthful in their label claims. However, developing this program will be complex and require substantial resources; therefore, it might not be a realistic goal for the near future . However, development of a pilot accreditation program might be possible and is worth pursuing. The pilot programs conducted by the Dreissena community might provide appropriate
models for these efforts.

3.2.2 Development of an Approved Laboratory Testing Program for Waterborne Pathogenic Protozoa – Ellen Braun-Howland, New York State Department of Health Environmental Biology Laboratories

Dr. Ellen Braun-Howland is the Director of the New York State Department of Health Environmental Biology Laboratories at the Wadsworth Center. Dr. Braun-Howland's laboratory participates in EPA's national approval program for detecting the pathogenic protozoa, *Cryptosporidium* and *Giardia*, in drinking and recreational waters. To provide a real-world example of developing and

operating an accredited laboratory program, Dr. Braun-Howland provided a detailed description of accreditation requirements for this program.

Cryptosporidium and *Giardia* are virulent human gastrointestinal parasites that cause severe gastrointestinal illness. Because these pathogens can be widely distributed in surface waters, they are regulated under the Safe Drinking Water Act, which falls under the purview of EPA. From the perspective of detection technology, the detection of these parasites poses many problems similar to those encountered when detecting *Dreissena* spp. larvae in plankton samples. The oocysts and cysts responsible for disease transmission are microscopic and require considerable technical expertise and specialized equipment for their detection and quantification. The current method for detection in water involves immunomagnetic separation of concentrated oocysts and cysts, coupled with fluorescent antibody-based detection using epifluoresence microscopy. These methods were developed in the 1990s and have since been standardized by EPA (Methods 1622/1623/1623.1).

Dr. Braun-Howland's group is also accredited by the National Environmental Laboratory Accreditation Program (NELAP) for the microbiological analysis of water for indicators of fecal pollution. As part of the approval process, laboratories seeking accreditation for analysis of either pathogenic protozoa or fecal indicator bacteria are required to participate in a proficiency testing program, which involves correctly analyzing sets of blind reference samples on a semiannual basis.

In addition to proficiency testing requirements, approval for analysis of waterborne *Cryptosporidiuim*, *Giardia* and/or fecal indicator bacteria requires that laboratories strictly follow Standard Operating Procedures (SOPs) associated with the applicable methods. Adherence to the method SOPs and documentation of associated quality assurance/quality control procedures are reviewed during regularly scheduled audits by outside accrediting bodies. According to Dr. Braun-Howland, while the process is "long and painful" it helps to ensure that testing is of high quality and legally defensible, thereby enhancing efforts to prevent epidemic outbreaks due to waterborne pathogens.

In discussion, the issues of cost and labor were raised. Beyond the initial considerable start-up costs, Dr. Braun Howland was not able to estimate laboratory costs since her laboratory, as a State Lab, does not charge for samples from public water utilities and they do not accept samples from private water suppliers. However, she indicated that the going rate for the analysis of single sample for analysis of pathogenic protozoa by a commercial laboratory is about $400. Dr. Braun-Howland estimated that in her laboratory about 20-25% of the labor is associated with QA/QC.
Dr. Braun-Howland's presentation gave great pause to all of the workshop participants. "Is this model appropriate for *Dreissena* spp. detection and monitoring?" was the unstated question on everyone's mind.

3.2.3 Laboratory Accreditation Standards: Perspectives from a Commercial PCR Diagnostics Laboratory – John Wood, Pisces Molecular LLC

Dr. John Wood is the founder and President of Pisces Molecular LLC (www.pisces-molecular.com). For the past 15 years, Pisces Molecular LLC has provided a variety of molecular-enabled diagnostic and research services focused primarily on aquatic organisms and ecosystems. Among the services offered by the company is early detection of *Dreissena* spp. in plankton samples by PCR-based methods. Clients of Pisces Molecular LLC include a variety of Federal and State wildlife agencies, private companies, zoos, wildlife researchers, and environmental organizations. Dr. Wood was asked to make recommendations from his perspective about structuring any eventual laboratory accreditation program for detection of *Dreissena* spp. in plankton samples. He summarized his perspective succinctly. Any accreditation process should be "easy, fast, cheap, and, most importantly, fair":

- The accreditation program should be easy to participate in and transparently understood. This can be achieved by defining clear and well-articulated certification standards that are not rigidly fixed to specific methods and mandates. Accreditation should be based on performance criteria, rather than strict adherence to a specific method or SOP. Dr. Wood pointed out that this was especially important for *Dreissena* spp. diagnostic methods that are still in development. Rigid SOPs will reduce new innovation, which is still needed in the arena of aquatic nuisance species management. Performance, rather than SOP-based standards, would allow for the incorporation of new technologies.

- The accreditation program should be fast. There should be a single accreditation authority and a mandate from all relevant management agencies to accept that accrediting authority. Dr. Wood pointed out that a cumbersome number of environmental accreditation programs and bodies already exist, making it an increasing burden on companies to maintain accreditation, especially small and relatively specialized companies such as Pisces Molecular LLC. This drives up costs and diminishes diversity of commercially available analytical services.

- The accreditation program should be relatively inexpensive. Costs should not be so high that they exclude or discourage small businesses from participating.

- The accreditation program must be fair. Rigorous and unbiased standards should be applied uniformly to all participants. Many laboratories involved in *Dreissena* spp. monitoring are Federal and State laboratories; therefore, it may be important that they not become the accrediting authority.

Following his overview description of what a *Dreissena* laboratory accreditation program should look like, Dr. Wood provided several recommendations for structuring such a program. Elements of the program would include SOPs that define sample tracking, handling, preparation, quality assurance (including appropriate controls for common laboratory artifacts), and specific reporting instructions. Dr. Wood raised an interesting question about whether there was a need to define what information should, and should not, be included in the report. Should there be reporting criteria for public versus private consumption?

Dr. Wood summarized his presentation by stressing that commercial laboratories will embrace accreditation standards if they can do it while making a profit.

3.2.4 History and Operation of the Society for Freshwater Science Taxonomic Certification Program (lessons learned for *Dreissena*) – John Morse, Clemson University

Dr. John Morse is an emeritus professor at Clemson University, a recognized world authority on the taxonomy of *Trichoptera* (caddisflies) and the co-chair of the Society for Freshwater Science' (SFS) taxonomic certification program (SFS-TCP). This award-winning program was developed by SFS in response to requests from Federal and State government agencies that depend on experts to identify aquatic invertebrates and communities as criteria for regulated environmental management purposes. The workshop organizers asked Dr. Morse to speak about the development of this program and to explore ideas for expanding it to include a subcomponent specific to the accurate identification of *Dreissena* spp. adults and larvae.

Dr. Morse began his presentation by describing the practical importance of accurate taxonomic identification in association with environmental management goals. As an example, Dr. Morse pointed out that the description of freshwater macroinvertebrate communities is increasingly used in U.S. regulatory and nonregulatory programs and that 95% accuracy of these assessments is generally required. He suggested that, for the purpose of detecting and monitoring aquatic nuisance species, similar criteria will likely be applied. However, a recognized impediment to realizing these needs is the lack of taxonomic expertise, as well as expertise that is not quality assured or well documented. Expert certification is necessary to ensure that a person has the competence to provide reliable taxonomic data. This, he pointed out, appears to be the problem with early detection and monitoring of *Dreissena* spp. Dr. Morse proposed that the SFS-TCP could offer assistance by developing a *Dreissena* spp. taxonomy expert certification program, and that doing so fits well with the charge of the SFS-TCP mission (www.nabstcp.com/default.aspx) and capabilities. However, Dr. Morse cautioned that an expert certification program alone would not ensure accurate information; it would complement laboratory QA/QC programs.

The SFS-TCP program includes training materials, trial exams, and graded exams. Training materials consist of online self-paced content, specimens that can be provided to registered participants, and the possibility of intensive onsite training short courses. Graded exams are fee based, and a well-defined appeal process is in place. Between the inception of the program in 2005 and 2011, 507 exams were administered and 310 certifications were issued. The SFS-TCP has a mandate to be self-funded and accrues funding through training and exam fees, as well as support from benefiting Federal and State agencies. Currently, the

program has annual funding commitments from the EPA and Environment Canada and receives some supplemental funding from the Society for Freshwater Science as the program continues to be developed.

With respect to the development of a *Dreissena* spp. identification program, Dr. Morse offered that SFS-TCP is in a position to and is willing to assist the Dreissena early detection community in developing a certification program. Once created, SFS-TCP could design and manage an examination for identification of *Dreissena* and other invasive species. Dr. Morse specified that what is needed from the *Dreissena* community was 1) a list of the taxa and life history stages for which people should be certified to be competent to identify, 2) a list of qualified experts (and their contact details) that are willing to volunteer to assist and 3) high quality digital imagery of all taxa and life history stages that need to be identified.

3.3 Component 2: Breakout and Plenary Sessions - Developing a *Dreissena* spp. Laboratory Standards Program

3.3.1 Breakout Sessions

Following the formal presentations, workshop participants dispersed into smaller breakout groups organized on the basis of their primary interest. Groups representing the interests of technical experts, resource managers, and stakeholders were established. Each group selected a discussion leader and note taker. Prior to entering into discussion, each group was asked to explore and discuss specific questions about the need and options for developing a *Dreissena* spp. early detection laboratory and standards program, and be prepared to present a summary to the entire group. Each group was asked to articulate from their unique perspective their opinions on the following questions:

- What is needed in terms of an accreditation/standards program?
- What are the immediate next steps required to advance the process?
- How, and by whom, should the accreditation program be managed?
- How would the accreditation program be paid for?

3.3.2 Plenary Presentations

Following approximately 2 hours of small group discussion, each group provided a concise summary in response to these four questions. The questions themselves, and the summarized answers, are presented below.

What is needed in terms of an accreditation/standards program?

Technical Experts. This group concluded that standard methods and protocols specific for each detection method were needed. However, it was concluded during the first workshop that defining universal standard methods is not currently possible, at least for IFC and molecular-based approaches. Therefore, equivalency of detection sensitivity and reliability is the most appropriate target for any eventual SOPs.

Resource Managers. This group concluded that performance-based laboratory and personnel testing are needed to achieve accepted certification standards.

Stakeholders. This group (the smallest group) concluded that what was most needed was a program that was understandable, transparent, trustworthy, and that clearly determined whether Dreissenid mussels are present in a system.

What are the immediate next steps required to advance the process?

Technical Experts. This group concluded that it was necessary to define quality goals (e.g., minimum detection limits) for early detection and quantitative detection, to articulate a plan for an accreditation program, and to develop cost estimates for such a program.

Resource Managers. This group concluded that the most important immediate step was to develop and implement a laboratory accreditation program that would include certification of expertise and SOPs.

Stakeholders. This group also concluded that it was critical to develop an accreditation program as the immediate next step. It was recommended that management authorities should commission the development of a laboratory accreditation program and, once it is developed, promote it.

How, and by whom, should the accreditation program be managed?

Technical Experts. Following a very short discussion, this group concluded that it was outside their scope of expertise to make any specific recommendation concerning the management of an accreditation program.

Resource Managers. This group also failed to reach a consensus concerning the organization and management of a laboratory accreditation program. However, it was suggested that the U.S. Department of Agriculture (USDA) might be an appropriate management agency for a *Dreissena* early detection laboratory accreditation program. It was noted that the USDA is currently independent of

Dreissenid issues and has experience with certification programs. The possibility of third party societies or an interagency professional group as a management agency was also discussed and seen as a viable option.

Stakeholders. This group held a long discussion concerning the establishment of management authority. The conclusions of these discussions were: (1) an interagency management commission should be established with the authority to manage the program, and (2) in addition to this commission, an independent oversight committee with the authority to arbitrate any disputes, should also be established.

How would the accreditation program be paid for?

Technical Experts. As was the case in their discussions concerning management structure, this group did not believe it was within their expertise to recommend how, or by whom, a laboratory accreditation program would be funded.

Resource Managers. This group spent their time brainstorming funding possibilities that ranged from fee-based cost recovery from stakeholders and participating laboratories, to funding from Federal agencies, corporate sponsors, or some combination of the above.

Stakeholders. This group concluded that there should be base Federal Government sponsorship supplemented by competitive grant funding, stakeholder dues, and fees from participating laboratories.

3.3.3 Plenary Discussions and Voting

Following the breakout sessions, the workshop was adjourned for the day. However, participants were encouraged to continue their discussions during the evening and to come prepared the following day to reach a general consensus on a roadmap forward.

The following day, the entire group met for one final time to review the accomplishments of the workshop and to discuss the recommendations each breakout group made during their plenary presentations the previous day. Dr. Frischer continued to moderate the session, and the text of each recommendation was reviewed and compiled into a single concise statement. When discussions were completed, support for each statement was assessed by a show of hands and recorded. A total of 27 workshop participants remained for the duration of this exercise. With the exception of one vote of abstinence, unanimous consensus was reached on each of the following statements.

What is needed in terms of an accreditation/standards program?

An understandable, transparent, trustworthy, performance-based system that accurately detect whether there are Dreissenids in a system.

Approved by unanimous vote (27)

What are the immediate next steps required to advance the process?

Initiate performance testing program in 2012 and articulate a plan for a long-term accreditation/certification program.

Approved by unanimous vote (27)

How, and by whom, should the accreditation program be managed?

Continue to use the Round Robin process as the testing program. Establish an independent advisory/review committee with management, technical, and stakeholder representation.

Approved by unanimous vote (27)

Organize a diverse commission with representation of management, technical, and stakeholder interests with authority to manage the program and an independent oversight committee composed of recognized technical experts with authority to arbitrate.

Approved by unanimous vote (27)

Pursue a partnership with the SFS, with the goal of establishing a program to certify the expertise of laboratory staff in the identification of all life history stages of Dreissenid mussels.

How would the accreditation program be paid for?

A detailed budget of anticipated costs should be developed, and diverse funding opportunities should be pursued. **Note: There was a lot of discussion, but it was not possible to reach a consensus on a specific funding plan. This was believed to be beyond the scope of the workshop authority.**

Approved by 26 votes; 1 abstained

The meeting was adjourned shortly after 12:00 p.m.

Appendix I

Workshop Participants

Last	First	Representing	Workshops Attended
Antwine	Mark	U.S. Army Corps of Engineers - ERDC	I & II
Baldys	Mick	U.S. Geological Survey	I & II
Braun-Howland	Ellen	New York State Department of Health – Environmental Biology Center Laboratories	II
Britton	David	U.S. Fish and Wildlife Service	I & II
Brock	Raphael	Texas Parks and Wildlife Department	I & II
Cannon	Norbert	Reclamation	I & II
Carr	Matthew	U.S. Army Corps of Engineers - ERDC	I & II
Churchill	Christopher	U.S. Geological Survey	I & II
Corbert	Terry	U.S. Army Corps of Engineers	I
Darling	John	U.S. Environmental Protection Agency	I
DeLeon	Ric	MWDSC	I & II
Euchner	Jason	IADNR	I
Ferrara	Carey	Frischer Biologicals	I & II
Fries	Loraine	Texas Parks and Wildlife Department	I & II
Frischer	Marc	Skidaway Institute of Oceanography and Frischer Biologicals	I & II
Hannam	Gwendolyn	U.S. Army Corps of Engineers	I & II
Heimowitz	Paul	U.S. Fish and Wildlife Service	I & II
Holdren	Chris	Reclamation	II
Hungerford	Tom	Texas Parks and Wildlife Department	I & II
Janicki	Erin	National Park Service (Glen Canyon National Recreation Area)	I & II
Jennett	Elysia	University of Arizona	I & II
Kelly	Kevin	Bureau of Reclamation	I & II
Kurtz	Victoria	Fluid Imaging Technologies	I
Lance	Rick	U.S. Army Corps of Engineers - ERDC	I & II
Lee	Chang	DWU	I & II
McGuire	Mandy	U.S. Army Corps of Engineers	I

McManon	Bob	University of Texas – Arlington	I & II
Merrill	Kaylani	EcoAnalysts	I & II
Misamore	Mike	Texas Christian University	I
Mobley	Brandon	U.S. Army Corps of Engineers (Fort Worth, Texas)	I & II
Morse	John	Clemson University	II
Nierzwicki-Bauer	Sandra	Rensselaer Polytechnic Institute – Darrin Fresh Water Institute, and Nierzwicki-Bauer Biologicals	I & II
O'Meara	Scott	Bureau of Reclamation	I
Phillips	Stephen	Pacific States Marine Fisheries Commission	I & II
Porter	Michael	USACE	I & II
Richards	David	EcoAnalysts	I
Rohde	Sasha	National Park Service (Glen Canyon National Recreation Area)	I & II
VanZee	Brian	TPWD	I
Wells	Steven	Portland State University	I
Wilson	Wade	U.S. Fish and Wildlife Service	I
Wood	John	Pisces Molecular LLC	I & II

Appendix II

Dreissena Early Detection Best Practices Technical Workshop Agenda – FINAL

Schedule Revised 2/6/2012

Tuesday, February 7, 2012

8:00	Gathering, Sign In *(coffee and breakfast available in the 1873 café and Market Square Cafeteria)*
8:30	Plenary Session in The Chambers Meeting Opening — Marc Frischer, Skidaway Institute of Oceanography, and Frischer Biologicals Housekeeping Issues — Mike Misamore, Texas Christian University QZAP program sponsor — David Britton, U.S. Fish and Wildlife Service
9:00	Meeting Agenda and Goal Setting — Marc Frischer, Skidaway Institute of Oceanography)
Expert Methods Presentations	
9:30	Cross Polarized Light Microscopy – Steven Wells, Portland State University *Using light microscopy and shell morphology to identify planktonic freshwater mussel larvae in early detection monitoring efforts for zebra and quagga mussels* Presentation and Discussion
10:30	Imaging Flow Cytometry – Erin Murchie-Janicki, National Park Service *Use of imaging flow cytometry (FlowCAM) for the detection of Dreissena larvae in plankton samples* Presentation and Discussion
11:30	Break
12:00	Molecular-Based Methods (PCR) – John Darling, U.S. Environmental Protection Agency *DNA-based detection methods in principle and practice* Presentation and Discussion
1:00	Lunch *(Market Square Cafeteria)*
Recent Technological Advances	
2:00	Imaging Flow Cytometry – Scott O'Meara, Bureau of Reclamation *Advancements in imaging flow cytometry (FlowCAM®) larval mussel detection technology and methodology*
2:30	PCR – John Wood, Pisces Molecular LLC *A combined ZM & QM qPCR assay and absolute quantification with a plasmid positive control*
3:00	Initial Charge for Breakout Groups – Recommendation for Best Practices
3:15	Breakout Group Discussions I. Cross Polarized Light Microscopy – Zeidman Conference Room II. Image Flow Analysis – Acuff Conference Room III. Molecular – The Chambers
Develop Specific Methodological Best Practices Recommendations	
4:00	Adjourn

Wednesday, February 8, 2012

8:00	Gathering, Sign In *(coffee and breakfast available in the1873 café and Market Square Cafeteria)*
8:30	Plenary Session in The Chambers – Recap of Tuesday and Charge for the Day – Marc Frischer, Skidaway Institute of Oceanography
Methods vs. Results – An Examination of Two Recent Data Sets	
9:00	2010 Round Robin Study – Marc Frischer, Skidaway Institute of Oceanography, and Frischer Biologicals Presentation and Discussion
10:00	Lake Texoma Data Set – Christopher Churchill, U.S. Geological Survey *USGS zebra mussel monitoring program for North Texas - Preliminary veliger data* Presentation and Discussion
11:00	Charge for Breakout Discussion and Continue Breakout Group Discussions I. Cross-Polarized Light Microscopy – Hillel Conference Room II. Image Flow Analysis – Acuff Conference Room III. Molecular – The Chambers
Complete discussion of best practices method recommendations and review general recommendation from Frischer et al. (2010) Round Robin Study report and expand from there.	
1:00	Lunch *(Market Square Cafeteria)*
2:00	Plenary Session in The Chambers *Unveiling the QZAP pilot testing program* – Sandra Nierzwicki-Bauer, Darrin Fresh Water Institute, and Marc Frischer, Skidaway Institute of Oceanography, and Frischer Biologicals
2:30	Discussion Presentation of Breakout Group Discussions Attempt to Reach Consensus of Recommendations
4:00	Adjourn

Appendix III

Dreissena Early Detection Laboratory Standards Technical Workshop Agenda – FINAL

Thursday, February 9, 2012 – Brown-Lupton University Union, The Chambers Room, 3rd Floor

8:00	Gathering, Sign In *(coffee and breakfast available in the1873 café and Market Square Cafeteria)*
8:30	Meeting Opening - Marc Frischer, Skidaway Institute of Oceanography, and Frischer Biologicals Housekeeping Issues - Mike Misamore, Texas Christian University and David Britton, U.S. Fish and Wildlife Service QZAP Program Sponsor - Paul Heimowitz, U.S. Fish and Wildlife Service Western Regional Panel Sponsor - Stephen Phillips, Pacific States Marine Fisheries Commission
9:00	Meeting Agenda and Goal Setting - Marc Frischer, Skidaway Institute of Oceanography, and Frischer Biologicals
9:30	Needs for Laboratory Accreditation (Certification Program) for *Dreissena* Monitoring. Mission of the Western Regional Panel for Laboratory Standards Program - Stephen Phillips, Pacific States Marine Fisheries Commission
10:00	QZAP Pilot Testing Program Unveiling Marc Frischer, Skidaway Institute of Oceanography, and Frischer Biologicals; Kevin Kelly, Bureau of Reclamation; Sandra Nierzwicki-Bauer, Rensselaer Polytechnic Institute
Model Programs – Case Studies	
10:30	Ellen Braun-Howland – New York State Department of Health – Environmental Biology Laboratories (Wadsworth) *Development of an approved laboratory testing program for waterborne pathogenic protozoa* (This presentation will cover the history and operation of a laboratory accreditation program for the detection and management of *Cryptosporidium* and *Giardia* in public water supplies.)
11:30	John Wood – Pisces Molecular LLC Laboratory Accreditation Standards – Perspectives from a Commercial PCR Diagnostics Laboratory
12:00 Lunch	
1:00	John Morse, Clemson University – Society for Freshwater Sciences' Taxonomic Certification Program *Presentation of the history and operation of the SFS Taxonomic Certification Program*. Lessons learned for *Dreissena*
2:00	Charge for Breakout Sessions – Developing a Laboratory Standards Program Breakout Discussion – Brain Storming Sessions Technical Experts – Hillel Conference Room Stakeholders – Acuff Conference Room Managers – The Chambers

	• What is Needed • What are the Next Steps • Management and Costs • Making it Happen
4:00	Plenary Presentation of Breakout Group Discussions Technical Experts – TBD Stake Holders – TBD Managers – TBD
5:00	Adjourn

Friday, February 10, 2012 – Tucker Technology Center, Conference Room, 1st Floor

9:00	Gathering, Sign In
9:30	Plenary Discussion Synthesize Recommendations and Plan for Advancing Laboratory Standards Program
12:00	Adjourn