A Comprehensive Report on the Workshop
‘Early Detection of Dreissena Mussels in the West’

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FINAL

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I. Executive Summary:

A one-and-one-half day workshop preceding a meeting of the 100th Meridian Initiative (21-22 January 2009) was convened for the purpose of making recommendations concerning the best available practices for the early detection and monitoring of Dreissena mussels in western waters in 2009 and beyond. Thirty eight representatives from several states, agencies, institutions, and other interests participated in the workshop.

The workshop agenda addressed four primary objectives. The first was to identify best practices for the detection at low concentrations the presence of Dreissenid larvae in plankton samples. The goal of this objective was primarily educational, with the state of the art of both microscopy and molecular approaches presented by experts. The second objective was also educational in nature and focused on discussing current monitoring and detection programs being utilized by different western states and regions. A limited number of representative presentations were made that outlined several ongoing detection and monitoring programs. These presentations were utilized to stimulate a broader discussion concerning the elements and feasibility of developing a comprehensive regional monitoring program. The goal of the third objective was to reach a consensus concerning the determination of whether Dreissena larvae are present in a body of water. Several proposals were put forward, discussed, and voted on. The final workshop goal was to develop specific recommendations for the 100th Meridian Initiative working groups charged with developing a comprehensive Dreissena mussel early detection and monitoring program for the western region during the 2009 season.

The recommendations derived from the technical workshop where at least a majority consensus was reached included:

1. For the purpose of a regional early detection and monitoring program the western region should include the 19 Western Panel States, Canada, and Mexico. Communication with other regions should be actively sought.

2. Substrate sampling should be continued. Samplers should be as simple as possible and maximize surface area and "edge" habitat. Samplers should be placed in areas thought to be at high risk and extend from the surface to the bottom with samplers at 10' depth intervals.

3. By an almost unanimous vote, veligers are considered to be present if their presence can be confirmed in a single plankton sample by at least one authenticated microscopy and one PCR assay.

   By ca. two thirds majority votes veligers are considered to present in a plankton sample if the presence is confirmed by two expert microscopists and/or by two different PCR assays.

   There was a consensus that a common set of terminology describing graduated levels (low to high) of infestation was needed.

4. Further research and development of PCR-based detection methods is required and worthwhile. Appropriate processing of plankton tow samples was recognized as a significant bottle neck for both microscopy and PCR detection approaches.

5. A standardized quality control and training program for labs involved in both PCR and microscopy veliger detection assays should be established.

6. The ongoing round robin study comparing PCR and Microscopy protocols should be continued.

II. Introduction

The purpose of this workshop was to bring together representatives from a variety of government agencies, universities and laboratories to discuss the issue of early detection of Dreissena mussels in the west. Zebra and Quagga mussels (genus *Dreissena*) are a Central Asian invasive species that pose a significant economic and ecological threat to western waters, water supplies, and water related infrastructures. A primary goal of the workshop was to provide technical advice to the 100th Meridian Initiative

group in their efforts to develop a regionally comprehensive Dreissena mussel monitoring program for 2009 and beyond. Specifically, during this workshop the issues of best practices for field sampling, sample analysis, criteria for defining levels of infestation, and the associated management practices were addressed. The goal was to bring representatives from different states and regions together to educate all involved on the current practices and procedures being used to detect, monitor, and manage Dreissena mussels in the West and to develop consensus, as much as possible, on these issues and to derive recommendations for the drafting of the 2009 Dreissena mussel early detection and monitoring plans. Although the issues discussed were wide ranging and have implications for long-term action, the focus was on developing recommendations for actions that could be implemented during 2009. The final goal of the conference was to provide recommendations on designing a comprehensive monitoring and early detection plan to the members of the 100th Meridian meeting that would convene directly after the workshop.

This report summarizes the four main objectives of the workshop. Each report section provides an introduction to the discussion topic, a synopsis of the information presented, a summary of the discussions that took place, and conclusions that were reached.

III. Objective 1: Identification of current state of the art detection and monitoring approaches.

Intended Outcome: Participants should understand the state of the art of cross-polarized light microscopy-based and PCR-based larval detection assays. Participants

should be familiar with survey/detection approaches for juvenile settlers and discovery of adult populations.

A. Field Detection of Juveniles Settlers and Adults

During this first session of the workshop five presentations were made to provide the participants with a comprehensive overview of current early detection best practices. The first presentation was given by Vicki Milano from the Colorado Division of Wildlife (CODW). Ms. Milano outlined the strategy used by the CODW to develop, implement, and sustain efforts to detect juvenile and adult Dreissena mussels. The CODW also monitors for larvae (veligers) and these procedures were also described. The CODW program is recognized to be among the most comprehensive Dreissena early detection monitoring programs in the Western US (Appendix I – Colorado Blue Ribbon Panel Report).

Initially, a risk assessment study was conducted to classify and identify Colorado waters that are believed to be at the highest risk of Dreissena mussel infestation. Qualitative risk was assessed by including water chemistry and nutrient chemistry information and by assigning a ranked assessment for the potential of introduction via recreational and other vectors. The risk criteria used by the CODW is included in this report as Appendix II. Each water body was assigned a ranking of one to four, with one indicating the highest risk. Based on this risk assessment, detection and monitoring efforts are guided by focusing on the most at risk water bodies within the constraints of available financial and personnel resources limiting the total number of water bodies that can be surveyed and monitored.

The most common methods used for detecting adult mussels has been to place suitable settlement substrates (substrate samplers) into the water and periodically examining them for the presence of mussels, conducing visual-based shoreline surveys, and visual surveys by SCUBA divers. In Colorado, substrates consist of plastic grids or PVC pipes that are attached to ropes and hung from buoys located in inconspicuous areas where the public are less likely to tamper with them. Substrates are deployed in locations deemed to be high risk areas (e.g. near areas where boats that may arrive from infested waters are launched). Substrates are placed from the surface to the bottom at 10 ft depth intervals. The use of ceramic flower pots is being considered in the future because of the how quickly the grids and PVC pipes are degraded. Other workshop participants volunteered that they have effectively used other materials as substrate samplers. For example, one participant described the use of "Sal's Tile," (A form of hard, cheap, porcelain tile) broken cement blocks, and plastic nursery trays with a corrugated pipe in the middle. Among the workshop participants there was general agreement that many substrates could be suitable, but that the important factor was that substrates should provide as much surface area and "edge" habitat as possible. Likewise, there was general agreement that braided nylon rope spaced with knots is the preferred rope type, again because this type of rope provides a substantial amount of surface area and edges for settlement of Dreissena mussel settlement. One workshop participant brought to the attention of the group that the California Department of Fish and Game has posted recommendations for substrate sampling of Dreissena mussels that might be useful for general adoption. In addition to substrate sampling protocols the California Department of Fish and Game has also published their protocols for conducting surface surveys and

veliger sampling. These protocols and a considerable amount of related information is available from the CA DFG website at (www.dfg.ca.gov/invasives/quaggamussel).

In Colorado, visual-based shoreline surveys are also routinely conducted. Technicians walk the shoreline examining rocks and wood in 1-3 ft of water looking for adult mussels. Another method of detecting adults is deploying SCUBA divers to examine lake beds at depths greater than 3 ft. However, due to high costs this approach is not commonly employed and is typically reserved for confirmation of possible sightings or to examine critical submerged habitats. In Colorado, substrate sample examination and shoreline surveys are conducted every two weeks for each high risk water body during the periods that mussels would be expected to be spawning.

To detect the presence of Dreissena larvae (veligers), horizontal and vertical plankton tows are collected and examined for the presence of veligers (see discussion below for methods). In Colorado waters are sampled when temperatures exceed 60 ° F, 3- 4 times per season and every two weeks for high and very high risk waters. The CDOW procedure is to conduct vertical tows from just above the bottom up to the surface. Distilled water is used to wash the materials from the nets from the outside in. The material is then transferred to a container and usually preserved in some way, often with ethanol. The percentage of ethanol that should make up the final sample is still a subject of discussion to be resolved through meetings of the technical experts.

CODW is utilizing both cross-polarized and PCR based methods for detection of veligers and current standards require positive identification to be confirmed independently by microscopic and molecular techniques. CODW is considering the adoption of an automated microscopy procedure that utilizes the FlowCam microscope

developed and marketed by Fluid Imaging Technologies. The CODW intends to purchase a FlowCam system in the near future and pursue its use to expedite microscopic examination of plankton samples for Dreissena veligers.

The presentation was concluded with the statement that all of the above methods are being employed to a varying degree in the ongoing effort to manage the invasion of Dreissena mussels in Colorado. However, methods have not yet been standardized fully and that further review is necessary to determine which substrate types are most effective, and to standardize plankton tow sampling and preservation methods. It is recognized within the CODW that additional research on the ecology and habitat condition preferences of zebra and quaggas in Colorado waters is needed to develop a more effective early detection and monitoring program.

B. Cross-Polarized Light Microscopy and PCR- based detection of Dreissenid larvae in Plankton Samples

The two most commonly used approaches for detecting Dreissenid veligers are Cross-Polarized Light Microscopy and Polymerase Chain Reaction (PCR) assays. The remaining four presentations during this section of the workshop were dedicated to these two analytical techniques. The presenters were Marc Frischer (Skidaway Institute of Oceanography, overview of topic), Denise Hosler (US Bureau of Reclimation, microscopy) and Steve Wells (Portland State University, microscopy and statistics), John Woods (PICES Molecular, PCR), and Sandra Nierzwicki-Bauer (Darrin Freshwater Institute, PCR vs microscopy). Both analytical methods have their own unique strengths and weaknesses, but both have proven effective at detecting Dreissenid larvae in water

and plankton samples. These methods were originally described in the scientific literature in 1995 (Johnson et al) and 2002 (Frischer et al), respectively. However, considerable practical experience by many different investigators have demonstrated that both methods can be technically challenging and sometimes the two methods yield different results, even when an identical sample is examined by both methods. Different regions and states have varying procedures on how the methods are used and what actions are triggered by positive results from either or both techniques. This section provides an overview of the two methods and discusses the advantages and disadvantages of each.

Both the microscopy- and PCR-based methods are designed to examine concentrated plankton collected with a plankton net from a suspect water body. Sampling typically involves concentrating plankton in the size range of Dreissena larvae (ca. 70-250 microns) using a net with a mesh size of 43 – 60 microns. Because the net is generally towed in the water (vertically or horizontally) to filter the large volumes of water necessary to capture Dreissena larvae when they are at low concentrations, these samples are often referred to as "plankton tow" samples. Typically 100–1,000 liters of water are filtered and concentrated up to 10,000-fold to 30–200 milliliters. The majority of the material collected in plankton tow samples consist of algae, non-Dreissena zooplankton species, and sediment. Therefore, a significant challenge for both microscopy and molecular Dreissena detection approaches is the definitive detection of larvae against a high background of other planktonic organisms and sediment particles.

Cross-Polarized Light Microscopy. Microscopic detection of Dreissena larvae in plankton tow samples is greatly facilitated by the use of cross-polarizing microscopy.

Because all bivalve larvae exhibit distinct birefringent characteristics, this approach allows bivalve larvae to be easily recognized under polarized light illumination. Under cross-polarizing light Dreissena larvae exibit a Maltese cross (x marks the larvae) and generally appear as bright white objects with a black cross (90° angle) while other plankton species are very dim under cross polarized light and do not exhibit the characteristic Maltese cross. Standard dissecting stereoscopic microscopes are easily and economically modified with the necessary polarizing filters or specialized microscopes can be purchased. In addition to the cross-polarizing microscope, only minimal additional materials are required for microscopic analysis of plankton samples. Other supplies and equipment include appropriate fixatives (usually ethyl alcohol), viewing slides, and possibly additional settlement concentrators. It is also useful if the microscope is equipped with photographic capability so that images of larvae can be photographed, shared, and studied by other experts.

Although Dreissena larvae are quite distinctive under a cross-polarizing light microscope, it is possible that they can be confused for other bivalve species that may be in a sample including the Asian Clam (*Corbicula fluminea*) and the dark false mussel *Mytilopsis* sp. Both these bivalve species can co-occur with Dreissena species. Additionally, to a poorly trained eye, the Ostracod crustaceans and even some sediment particles may be mistaken for Dreissena larvae. Therefore, it is of critical importance that any technician examining plankton tow samples for Dreissena larvae be well trained, experienced, and familiar with other particles that may possibly be mistaken for Dreissena larvae. To increase the likelihood of correct identification the technician should be able to easily switch between cross polarized and non-polarized illumination to

analyze further diagnostic characteristics. For instance, *Corbicula* veligers may look like D shaped Dreissenid veligers, but *Corbicula* are much larger and their size ranges do not overlap. Ostracods have distinct appendages that can be identified under non-polarized light. In general, if an object exhibits a Maltese cross under cross-polarized illumination, the object should also be examined under normal light to identify other diagnostic morphological features. In addition, high quality digital images should be taken of any suspected positives to be sent for outside opinions.

Additional analytical problems in detecting and identifying Dreissena larvae can arise if the samples are poorly preserved or if they contain exceedingly high concentrations of non-Dreissena plankton. Poor preservation can result in the dissolution of veliger shells resulting in false negative. Shell dissolution is common if the sample becomes even slightly acidic and is stored for any period of time. Experts at Portland State University recommend not using denatured alcohol although there is not yet a concensus among experts regarding the optimal concentration of alcohol. In the literature concentrations of 25-95% ethanol have been successfully utilized. Extremely high plankton concentrations in samples often obscure the detection of Dreissena larvae even under cross-polarizing illumination. When plankton concentrations are too high it is necessary to dilute the sample (usually in distilled water) which greatly increases the amount of time required to examine the sample. Finally, it cannot be over emphasized that plankton communities vary between water bodies and with season, thus microscopist technicians must be well trained and familiar with plankton communities and plankton successional cycles to be able to examine and detect larvae in a diversity of plankton samples that they are likely to encounter.

Polymerase Chain Reaction (PCR). PCR is a process that allows for the *in vivo* amplification of specific genes fragments. Since gene fragments unique to Dreissena larvae can be targeted by a specific PCR assay, this approach can be utilized to detect the presence of Dreissena larvae in a plankton sample, even if they are at very low concentrations. Theoretically, PCR assays provide several distinct advantages compared to cross-polarized light detection strategies including 1) increased sensitivity, 2) increased species specificity, and 3) the ability to process a much larger number of samples in the same amount of time needed to process microscopy samples. However, the realization of these advantages still requires considerable research and development.

PCR is a technique widely used in molecular biology. It derives its name from one of its key components, a DNA polymerase used to amplify a piece of DNA by *in vitro* enzymatic replication. As PCR progresses, the DNA generated is used as a template for replication. This initiates a chain reaction in which the DNA template is exponentially amplified. With PCR it is possible to amplify a single or a few copies of a piece of DNA to generate millions or more copies of the DNA piece. This amplification makes it possible to simply detect the presence of a diagnostic genetic characteristic; for example, a gene unique to Dreisenna species. Almost all PCR applications employ a heat-stable DNA polymerase. This DNA polymerase enzymatically assembles a new DNA strand from DNA building blocks, the nucleotides, by using single-stranded DNA as a template and DNA oligonucleotides (also called DNA primers), which are required for sequence-specific initiation of DNA synthesis. The vast majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample to a defined series of temperature steps. These thermal cycling steps are necessary to physically

separate the strands (at high temperatures) in a DNA double helix (DNA melting) used as the template during DNA synthesis (at lower temperatures) by the DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions. In the case of Dreissena-specific PCR assays, single stranded DNA primers (oligonucleotides) are designed that are specific for either *D. polymorpha* (zebra mussel), *D. bugensis* (quagga mussel), or that are capable of specifically amplifying both species. To date, primers have been designed to target several different genes present in these species including the small subunit RNA (18S rRNA) gene, the Ribosomal Internal Transcribed Spacer sequences (ITS 1 & 2), and the mitochondrial Cytochrome Oxidase I (COI) gene.

The lab infrastructure and materials required for PCR is more extensive (and expensive) than is required for cross-polarized microscopy. However, PCR capability is currently available in a very large number of research, clinical, and commercial diagnostic laboratories and the PCR technique is relatively easily learned and is regularly incorporated into classroom labs in undergraduate and even high school biology programs. Thus, there is a large availability of laboratory infrastructure and personnel capable of performing Dreissena-specific PCR assays.

In practice, PCR detection of Dreissena larvae in plankton samples is subject to many possible types of error that may result either in failure to detect larvae when they are present or that indicate the presence of larvae when they are not actually present in a sample. One important factor that may result in failure to detect larvae when they are present or that reduces the detection sensitivity of PCR assays is that practicality requires

that only a relatively small subsample of a plankton tow sample can be assayed whereas it is generally feasible to examine an entire plankton tow sample microscopically. This limitation has been recognized by a consensus of the workshop participants as being the most critical limitation of Dreissena-specific PCR assays and should be the subject of a concerted and focused research effort. False negatives can also be obtained if substances that co-purify with DNA from the plankton sample inhibit PCR amplification of Dreissena DNA. Several substances present in plankton tow samples including mucopolysaccharides, humics, and metals have been shown to be potent inhibitors of PCR. However, most assays that have been developed are relatively effective at removing inhibiting substances and effective assay controls are included to rule out the possibility of false negatives due to the presence of PCR inhibitors. Finally, false negatives can be obtained due to poor preservation resulting in the degradation of DNA containing tissues. The proclivity to obtain false negative results by PCR is believed to be the primary reason why the most frequent observation of discrepancies between PCR and microscopy results from the identical plankton tow sample is the detection of veligers by microscopy but failure to detect by PCR. In a recent analysis of 372 plankton tow samples collected from western waters in 2007 and 2008, PCR and microscopy assay yielded discrepant data. In this data set, of the 29.3% (109) of the samples where the results of PCR and microscopy were conflicting, 26% were samples in which veligers were detected by microscopy but were not detected by PCR (Figure 1).

PCR \ Microscopy	+	-
+	39	11
-	98	224

Figure 1. Results of PCR and cross-polarized light detection of Dressenid mussel larvae from 372 plankton samples collected from Western waters in 2007 and 2008. Of the samples examined, in 224 (60.2%) veligers were not detected by either PCR or microscopy, in 39 (10.5%) of the samples veligers were detected by both PCR and microscopy. Discrepancies in the detection of Dressisenid larvae by PCR and microscopy were observed in 109 (29.3%) plankton samples. Of these samples the majority (98) resulted from detection by microscopy without detection by PCR and 11 resulted from detection by PCR but not microscopy.

Alternatively, false PCR positives can be obtained if the sample becomes contaminated with even the minutest amount of Dreissena DNA or if the primers being used amplify non-Dreissena DNA, though the primers available today appear to be highly specific. However, although great strides have been made to limit these types of errors, it is recognized that additional research is needed to further develop, optimize, and validate Dreissena-specific PCR assays that are effective and practical under realistic field conditions. Currently there is considerable ongoing research in this area, but funding of this type of research is limited and greatly needed.

Both cross-polarized microscopy and PCR methods have advantages and disadvantages. Microscopy is currently the more established and common method, while PCR has the potential to become the more time efficient, specific, and sensitive method. Workshop participants agreed that standards for plankton sampling, sample preservation, lab quality control, and data reporting are needed. The experts in both microscopy and PCR have agreed to hold technical workshops to discuss and attempt to attain consensus on these issues.

C. Microscopy vs. PCR: Initial Results of a Double Blind Round Robin Study

Because analytical results from nearly 400 plankton samples analyzed during the 2007 & 2008 field seasons revealed a relatively large number of samples where microscopy and PCR results were not in agreement (Figure 1), in late 2008 a preliminary study was undertaken to determine the source of these discrepancies. The objective of this study was to determine the amount of variability between Microscopy & PCR results that could be attributed to the analytical procedures. The preliminary conclusions from this study were presented during the workshop by Professor Nierzwicki-Bauer from the Darrin Fresh Water Institute. The results presented represented the first phase of the conceived study which, when complete, would also examine errors associated with processing complex concentrated plankton samples (Phase II), and errors associated with sampling procedures (timing, frequency, location) (Phase III). The experiment was designed by Kevin Kelly (US Bureau of Reclamation), Marc Frischer (Skidaway Institute of Oceanography), and Sandra Nierzwicki-Bauer (Darrin Fresh Water Institute). The study was unfunded and all participants volunteered their time, expertise, and resources.

In phase I of the study participating microscopy and PCR labs were sent water samples that had been spiked with known numbers of zebra mussel (*Dreissena polymorpha*) veligers and asked to report whether larvae were present, and if possible, to quantify larvae and determine whether *D. polymorpha* and/or *D. bugensis* were present. Larvae-free water from Lake George, NY was spiked with *D. polymorpha* larvae collected from Lake Champlain, VT. All samples were collected and prepared by Sandra Nierzwicki-Bauer and her team at the Darrin Fresh Water Institute (part of the Rensselaer Polytechnic Institute) in New York. Zebra mussel veligers (*D. polymorpha)* were collected and quantified using microscopy. Known numbers of veligers were distributed into 40 mL of veliger-free lake water (25% ethanol). Twelve replicate sets were made, with five samples per set. The concentrations were 0, 1-5, 20-40, 50-70, and >100 veligers per sample. Five sets were sent to microscopy labs, which included Fluid Imaging Technologies in ME (H. Nelson), U.S. Fish and Wildlife Service in Texas (D. Britton), Skidaway Institute of Oceanography in Georgia (M. Frischer), Bureau of Reclamation in Colorado (D. Hosler) and Portland State University in Oregon (M. Sytsma and S. Wells). Another five sample sets were sent to PCR labs at the Metropolitan Water District in CA (P. Rochelle), Pisces Molecular in Colorado (J. Wood), Skidaway Institute of Oceanography in Georgia (M. Frischer), Bureau of Reclamation in Colorado (K. Kelly), and U.S. Geological Survey in Washington State (R. Rodriguez). Each participating lab was sent an instruction letter and the uniquely coded samples. A representative copy of the invitation letter is included as Appendix III.

Due to issues of sample integrity (leaking that occurred during shipping) a total of 24 samples were analyzed by microscopy and 20 by PCR. With respect to

presence/absence detection, 23 of the 24 (96%) microscopic analyses agreed with expected results while 17 of 20 (85%) of the PCR analyses matched expectations (Figure 2).

Actual

A

Observed	Actual +	Actual -
+	19	0
-	1	4

B

Observed	Actual +	Actual -
+	14	1
-	2	3

Figure 2. Comparison of veliger detection during the round robin study samples by (A) cross-polarized light microscopy and (B) PCR. Of the 24 test samples examined by microscopy all but one were identified as expected. The single discrepancy occurred in a sample which contained 1-5 veligers (actual) but no larvae were detected. Of the 20 samples examined by PCR all but 3 were identified as expected. In one case veligers were detected in a sample that did not contain larvae and in two cases samples that contained larvae (0-5) larvae were not detected.

In both microscopy and PCR analyses, all errors occurred in samples that contained the lowest concentration (1-5) or no veligers. Quantification of larvae by microscopy was only semi-quantitative, especially when higher numbers of larvae were present (Figure 3). Variability between analytical labs was also documented, with PCR labs exhibiting a higher amount of variability then microscopy labs (Figure 4). In general the phase I round robin study illustrated and quantified analytical error associated with both microscopy and PCR approaches. Presence/absence detection by microscopy out performed PCR by about 10% with respect to absolute accuracy. Inter-lab analytical

variability of PCR analyses were about twice the rate of microscopy analyses. However, it was also evident that both approaches had the potential to be 100% accurate.

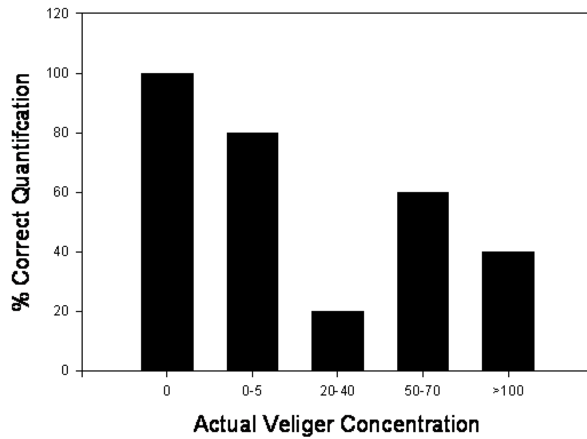

Figure 3. Quantification by cross-polarized microscopy of Dreissena veligers in the round robin study. The accuracy of quantification, except at the lowest concentrations, consistently underestimated actual veliger concentrations.

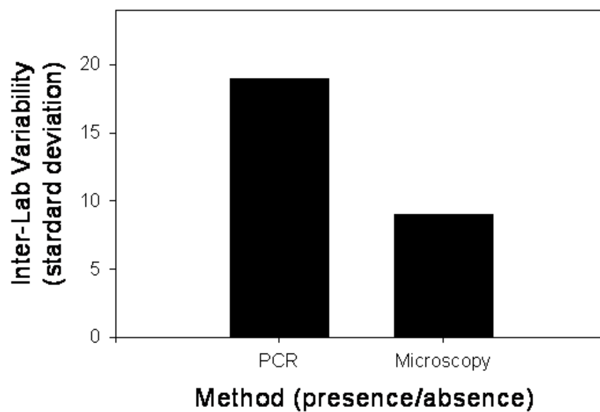

Figure 4. Standard deviation of presence/absence veliger detection results from independent laboratory analysis by PCR and cross-polarized microscopy examination of round robin study samples. Five PCR and microscopy labs examined replicate sample sets. Interlab variability among PCR labs was higher ( ) then observed among microscopy labs ( ). Two out of the five PCR labs correctly identified larvae in all samples and four out of the five microscopy labs correctly identified larvae in all samples.

Several conclusions and recommendations can be made from this experiment. First, this study revealed considerable analytical variability between labs and methods, despite the simplified nature of the plankton samples that were examined. Both methods had analytical errors, and the error rates were roughly comparable for both methods. The goal is for all labs to have 100% accuracy with both methods. This is not impossible as two labs involved in the study reported back 100% correct results. In the future analytical methods and data reporting should be standardized between labs as much as possible. At

the upcoming technical meetings between representatives at the microscopy and PCR labs hopefully many of those issues can be resolved.

Pending funding, phase II of the study is scheduled to begin during the late spring or early summer of 2009. The focus of the phase II study will be to compare the efficacy of Dreissenid larvae detection by microscopy and PCR at low concentrations (0-5 per ml of concentrated plankton sample) in the presence of natural plankton from Western waters.

IV. Objective 2: Identification of current regional practices for early detection, monitoring of Dreissenid mussels.

Intended Outcome: Familiarize all participants with ongoing early detection and monitoring programs. Recognize the priorities and limitations facing each state.

To address Objective 2 goals, three presentations were provided by representatives from western states. Larry Dalton (Aquatic Invasive Species Coordinator, Utah Division of Wildlife Resources) provided and overview of Utah's Dreissena mussel monitoring and action plan. Utah is a state where mussel and/or larval sightings have been reported but which does not have established populations. Allen Pleus (Aquatic Invasive Species Coordinator, Washington State Department of Natural Resources) provided an overview of the actions taking place in Washington State. Mussel infestation has not yet been reported in Washington State. The third presentation was provided by Ricardo DeLeon (Metropolitan Water District of Southern California). Unlike Utah and

Washington State, California is currently dealing with a major infestation of Dreissenid mussels.

Utah has in place a comprehensive aquatic invasive species (AIS) program that coordinates the efforts of a variety of agencies and stakeholders. The establishment and implementation of the program resulted from being able to convince the state legislators of the economic value in establishing a proactive monitoring and protection action plan to prevent a successful invasion of Utah. Dalton and his team were able to convince the Utah State legislature by documenting the potential costs of managing a zebra and quagga mussel invasion including maintenance, repair, loss of revenue and containment strategies. In Utah, like many western states, there are limited water supplies and a large number of primary pipelines and canals. If mussels were to start populating these pipes there could be a major decreases of water flow to irrigation systems, electrical plants, pipes for industrial purposes and water treatment facilities. Zebra and quagga mussels also have significant ecosystem impacts. Anglers and boaters bring in over $708 million dollars annually to UT, and if they have a negative experience, they won't come back. The mussels compete with many fish species for food, and can cause decreases to average fish size and health. In addition, Utah lowers water levels at certain times of year, and exposed, dying mussels would give off a foul odor and leave beaches full of sharp shells.

Based on his research, it was estimated that if an infestation occurred in Utah of comparable magnitude to the zebra mussel invasion in eastern states, it would cost Utah over $15 million a year to deal with the problem. CA, NV and AZ have already spent $10 million in their first year of dealing with their infestation of quaggas. Larry's team

submitted a proposal to the UT legislature explaining all the statistics and requested funding for plan to keep Dreissenid mussels out of the state. Senate Bill 238 was passed that provides $1.4 million annually for interdiction and supports 47 employees.

Utah's AIS plan for invasive Dreissenid mussels includes a prioritized risk assessment similar to that used in Colorado for all waters, public education and outreach, and rapid response plans. High risk waters are monitored for larvae and by using substrate samplers, boat inspections and SCUBA diver aided visual inspection. A water body is put into "containment" mode if there is any confirmation of a veliger, and is labeled a "watch" water. A water body is officially "infested" if either an adult is found or a veliger is confirmed by microscopy and by PCR. When a water body is in containment mode, sampling increases, all boats going in or out are rinsed with scalding water and dried for 7 days in the summer and 18 days in the spring or fall. At the time of the presentation only one lake has been "infested" (Electric Lake) although eight additional lakes have been placed on the "watch" list due to a single detection of a veliger larvae.

Utah is model for how a state can communicate with its legislature for funding and put in place a quality program that has so far appeerently effectively prevented the invasion of Dreissena mussels into the state. Colorado did not give presentation at this conference, but their management plan is similar to Utah's and has had similar results. Utah's motto for dealing with the mussels is "Forever keep them out, or forever live with them."

Washington State has developed a management plan for the 70 major water bodies in its jurisdiction. Like other states, Washington State has developed a risk

assessment and a rapid response plan as part of their management plan (http://wdfw.wa.gov/fish/nuisxsum.htm)**.** Additionally, Washington State has put into action a large scale effort to educate related employees and the public on this issue. They employ boater surveys, vessel inspections, AIS check stations, water sampling and a special July 4th emphasis patrol. Due to a lack of analytical resources, all plankton samples are outsourced for identification of larvae microscopically. Plankton samples are collected by volunteers. All boats at the Washington State port of entry are inspected. Currently there have been no confirmed veligers or adults in any sampling efforts from this state.

Ricardo DeLeon (California) provided an overview of the Western areas that already have serious infestations. Quagga mussels were first discovered in abundance on January 6th, 2007 in Lake Mead, Nevada. California, Nevada and Arizona all have water bodies that have a confirmed presence of adult quagga populations. For these regions, effort has switched from detection to containment and control. The Southern California Metropolitan Water District has conducted vulnerability assessments of infrastructure, and applied research on best practices for controlling adult populations. Siphons, aqueducts canals, trash racks and other infrastructure are carefully monitored and inspected. A variety of control methods are used. Approaches include the use of disinfectants, oxygen deprivation, chemical additives, physical removal, drying, and infrastructure surface coatings. The southwestern examples show the damage mussel invasions can cause and demonstrate how serious this issue is to all western states.

During the discussion of Dr. DeLeon's presentation, a number of issues were raised about standardizing detection procedures, response actions, and the unique

challenges facing each different state. The following were recommendations from the meeting participants. It was expressed that there should be standard protocols for sampling based on a water area's parameters (size, water type, risk etc) that everyone could follow. If a state wanted to do more monitoring that would be fine, but there should be a recommendation for the minimum amount of monitoring needed. If any veligers are confirmed, monitoring should greatly increase at all levels. If an area has adults then it's probably already too late to stop them. In addition, it was also recommended that standard sample preservation protocols should be adopted. A common sample preservation protocol will allow more effective inter-lab and inter-state comparison of results.

Increasing turnaround time from sample collection to results would greatly aid management agencies. Since the number of samples will likely increase over the next several years, it is clear that there will be a need for additional infrastructure and help in analyzing samples. It was suggested that the quickest way to do this would be to put efforts into training a large number of people in microscopy, since most labs already have most of the infrastructure needed. Others thought that while PCR is more expensive and complex, money needs to go into funding PCR research because it has the potential to be a much more efficient and specific method. Standards for PCR sensitivity and specificity should be in place. All fish health monitoring sites have PCR capabilities and could potentially be of assistance, although this isn't the type of monitoring they usually do, and they may not be amenable to this idea. It was also added that it is often too risky for a management agency to use anything but certified labs for their samples because of the political and media scrutiny they face.

In outreach issues, boaters get confused by the different rules in place in different states, and this type of outreach would be more effective if policies were standardized. Finally, Utah recommended to all states the importance of communicating now with upper level managers and politicians about the future costs of not funding early detection and the probability of finding veligers based on the resources given. (Utah's proposal to their legislature is available as example- contact Larry Dalton).

V. Objective 3: Identify the most important criteria for deciding that a body of water is infested with zebra/ quagga mussels.

Outcome: Reach consensus among participants (managers and scientists) about when a water body is considered to be mussel infested. Propose a scaled (Green to Red) system of zebra mussel risk that could be linked to specific management actions. Specific management actions were not discussed at this workshop, but they were a topic at the 100th Meridian that followed the workshop.

During this component of the Workshop, a general discussion about how to decide 1) when a water body clearly has an indication of Dreissenid mussel presence and 2) what are appropriate triggers for implementing specific management actions was conducted. As a straw man proposal, an alert system similar to that employed by the Department of Homeland Security to warn of terrorist threat levels was suggested. However, during the discussion it was quickly apparent that a common definition of risk levels was required and therefore the bulk of the discussion focused on articulating a common set of terminology for describing graded levels of infestation, and what was

needed to develop a consensus on the analytical tools and results that would be required to confirm risk levels.

It was initially suggested that it might be prudent to regard every water body as though it has Dreissenids from a management standpoint since early detection is quite difficult and expensive. However, others pointed out that this is probably not a realistic approach due to lack of resources. There was general agreement that if adults are found growing on substrates or infrastructure, than a water body was definitely infested. If adults are there however, it is likely too late to stop a population from establishing. It was also added that if veligers are found consistently then there must be adults nearby even if they haven't been found yet. The main focus of the discussion concerned the presence of veligers, since it was generally recognized that detection of low concentrations of larvae provides the earliest possible detection level when it is possible to attempt eradication management action. Participants were also reminded though that first findings have occurred through substrates and from public outreach, and if all the resources go into sampling and analysis for veligers, a critical part of the process is being neglected.

A series of terms were presented, but not talked about in detail due to time constraints. It was agreed that additional discussion would take place at the 100th meridian meeting, and that it was extremely important that a common language was available for states to communicate with one another about infestation levels. The water condition definitions offered during this meeting were as follows:

-Not tested (no monitoring has occurred)
-undetected (no evidence has ever been found to suggest presence of dreissenids)
-exposed (indirect evidence such as a boater survey that suggest a pathway)
-suspect (direct evidence such as veliger confirmation by only one method or finding a contaminated boat in the water)
-detected (veliger confirmed by microscopy and PCR)

-infested (signified by in situ reproduction).

There was general consensus that elevated management action should be taken whenever a confirmed larvae is found. A series of votes were taken to decide what results are needed analytically to confirm the *presence* of a veliger from a plankton tow sample. These votes actually took place during the Objective 4 discussion, but they more closely related to the Objective 3 outcomes, so are included here. The yes votes signify their agreement that the proposal confirms the presence of a veliger.

1. One detection event verified with PCR and microscopy
   Vote: Almost unanimous

2. One detection event that is verified by microscopy by two experts with multiple, high-quality images.
   Vote: 24 yes, 14 no

3. One detection event that is verified by PCR by two different assays.
   V***ote: 24 yes, 11 no, 3 abstentions***

An additional vote was taken to see if the participants thought that two separate confirmed detections (using proposal 1) were needed to declare presence. Only two people voted yes, and everyone else agreed presence could be declared with a single detection event. Several participants noted that using proposals two and three were not possible politically, because of the pressure on them to provide highly convincing proof of their results to non-scientific entities. The general consensus was that to identify a veliger in a sample and trigger management action, which is one of the most critical early detection tools for western states, the larvae needs to be confirmed by microscopy and PCR.

VI. Objective 4: Outline a comprehensive monitoring and early detection plan that can be used to draft the 2009 plan at the 100th Meridian meeting.

Outcome: Produce a draft of an early detection and monitoring plan that will facilitate its draft at the following 100th Meridian meeting.

To jumpstart the process of reaching a concensus concerning the outline of a Western region comprehensive monitoring and early detection plan, Elizabeth Brown (Colorado Division of Wildlife) provided and overview presentation that summarized the current status of the western invasion of zebra and quagga mussels. During her presentation Ms. Brown reviewed the first discovery of quagga mussel in Lake Mead and their subsequent discovery and downstream expansion. At the time of her presentation the presence of Dreissena mussles have been confirmed in 28 locations in California, Lake Havasu and Lake Pleasant in AZ, and several other locations downstream from these locations. Colorado has seven waters with confirmed veligers, though Lake Pueblo is the only one where they are finding them consistently and fear there must already be an adult colony. Utah only has one water body, Electric Lake, that has a confirmed veliger. There are 8 eight other waters that had veliger samples confirmed by one method but not the other. No other states have confirmed Dreissenids in their waters. However, there are many states that do not yet have well developed monitoring programs and their waters are fairly untested.

The remainder of the time allocated for this discussion was dedicated to reaching a consensus of the boundaries of the "Western Region", discussing unresolved issues, and talking about any monitoring and detection input that hadn't been heard yet.

After the presentation there was a discussion on what the definition of the "western region" should be for the purposes of coordinating and cooperating on the issue of early detection and monitoring of Dreissenid mussels. Three management groups were

brought up that are involved in this issue: the 100th Meridian Initiative, the Western Regional Panel (WRP), and the Western Region Fish and Wildlife. It was agreed that once the recommendations from this meeting were finalized they should be sent to the chairs of all three groups who should then meet to discuss them. There are 19 states included in the WRP, and it was brought up that there are 4 states, Missouri, Iowa, Arkansas and Louisiana, that have downstream connectivity to WRP states. Participants also brought up that states in Canada and Mexico have shown interest in being included in communicating on this issue. A vote was taken and it was decided that the Western Region would include the 19 Western Panel States as well as Canadian and Mexican regions that wish to be involved.

Larry Dalton added at the end of this discussion that another state that has an aggressive and comprehensive early detection and monitoring plan is Minnesota, which has for the most part kept out Dreissenids for over 20 years. Minnesota has an excellent website that describes what they've done, which Utah has used as a model in developing their program.

While the outcome of Objective 4 was not able to be realized in real time during the workshop, this report will provide a draft for the 100th Meridian Initiative to produce a 2009 Western Region early detection and monitoring plan. The conclusion of this document is a comprehensive list of the conclusions and recommendations made by the participants of this workshop. Not all of these may be able to be implemented in the 2009 plan due to limited resources, but they are all worthy of mention. The list is categorized by subject, and includes sampling and monitoring, analysis, and communication and coordination issues.

Sampling and Monitoring

1. Standard protocols are needed for plankton tows based on an area's basic parameters. (eg. Lake vs. river, shallow vs. deep water)
2. Standard protocols are needed for preservation and handling of plankton samples.
3. A standard protocol is needed for developing a risk assessment for a water bodies
4. Plankton samples should be taken from high flow and turbulent areas when possible, such as around dams, inflows, and outflows. Inside a dam is also a convenient place to obtain a sample.
5. Substrates should be placed at 10 ft intervals through the water columns from the surface to the bottom. Braided nylon rope with knots in it should be used. A variety of substrates can be employed, but one should attempt maximize strength, ease of handling, surface area and edge habitat. Some substrates that have been used are: dark pvc pipe, flower trays, flower pots, bricks, broken cement blocks and just rope by itself.
6. Sarah Mueting at UNLV should be contacted for her substrate research results.
7. States should keep in mind that first finding have been discovered through substrates and public outreach, and that these strategies need funding too. Public outreach should be utilized as much as possible.
8. Basic protocols for diver surveying are needed. Areas looking for local divers can try contacting their police departments.
9. Funding should be allocated for research to advance the understanding of Dreissenid mussel ecology in order to better predict where adults and veligers will be located.

Veliger Analysis

1. There needs to be an investment in infrastructure, both for microscopy and PCR, as the number of samples is only going to increase and current labs aren't able to keep up with demand.
2. Laboratory turnaround time for samples needs to be improved.
3. Result documentation and reporting formats need to be standardized. Results that are sent back to the originators of the samples should include detailed documentation of the procedures used to analyzed the samples. Documentation should include analytical protocols and results including micrographs, taxonomic descriptions of identifying features, gel images, genetic sequence data.
4. There needs to be standardized procedural methods between labs, possible leading to lab certifications. For PCR the focus needs to be on sensitivity and specificity.
5. There was a consensus by a nearly unanimous vote that a presence of a veliger can be confirmed if it has a positive PCR *and* microscopy result. About 2/3 of the participants voted yay that presence can be declared by two positive PCR assays *or* two positive microscopy confirmations from separate labs.
6. Experts in both PCR and microscopy should each convene for technical workshops to discuss issues of standardization between labs.

7. The Round Robin study should continue and Phase II should begin as soon as possible.
8. Funding should be allocated for research to improve the efficiency of the PCR process, which has the potential to greatly reduce labor costs and turnaround time.

Communication and Coordination

1. Managers of different states and river basins should communicate with one another about their management plans and water body infestation levels, especially when downstream waters affect a different state or basin.
2. A common set of terminology to describe graded levels of infestation needs to be created.
3. By a vote of the participants, the definition of the "Western Region" will be- the 19 western panel states, which included Alaska and Hawaii, as well as regions of Canada and Mexico that wish to be involved.
4. It is important to actively communicate and gain insight from eastern states, specifically states that have been successful in stopping invasions, such as Minnesota.

The recommendations from this document and the results from the technical workshops will aid the 100th Meridian in their effort to produce the 2009 and future plans. With guidelines for success, coordination between different management, and effort on the part of all western states, hopefully this noxious invasive species will forever be kept out of the currently Dreissenid- free and beautiful waterways of the west.

VII. Cited References

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